ROOT LIFESPAN AND OVERWINTERING STRATEGIES FOR TWO WETLAND SPECIES, *SAGITTARIA LATIFOLIA* WILD. (ALISMATACEAE) AND *CAREX CRINITA* LAM. (CYPERACEAE), IN CONTRASTING CLIMATE ZONES.

by

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Abstract

In northern wetlands, roots of some perennial wetland plant species senesce in the autumn, while other species maintain their roots over the winter. Some of these species extend in their distribution to warmer climates. This research investigates the effects of growing season length on root phenology of two species with such contrasting overwintering patterns. *Sagittaria latifolia* (autumn-senescing roots) and *Carex crinita* (overwintering roots) were investigated near the northern (46°N) and southern (35°N) edges of their distribution in North America. *C. crinita* maintained >75% of its roots alive in both locations, without changing its overwintering strategy. Roots of *S. latifolia* senesced completely in the autumn in the north, whereas, in the south some roots (~10%) remained alive throughout the winter. This suggests that complete root senescence before the winter is an adaptation to long winters, and may be unnecessary under short mild winters. Nevertheless, the species maintains the short-lived root strategy regardless of the winter severity.

Keywords: root phenology, growing season length, root senescence, wetland, overwintering roots, autumn-senescing roots.

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1 Introduction

Senescence is an important process of nutrient remobilization to optimize nutrient usage under adverse conditions, especially in seasonal climates with considerable annual turnover of biomass (Noodén *et al.* 1997; Gill and Jackson 2000; Thomas 2013). The seasonal pattern of biomass turnover in plants allows adaptation to the various environmental conditions along a gradient of a growing season length (Gan and Amasino 1997; Thomas 2013). A commonly cited example is that of deciduous trees in temperate regions which adapt to an unfavourable season by senescing their leaves to avoid damage during the winter and compensate for their annual leaf loss with high photosynthetic rates (Lechowicz 1984). This strategy allows deciduous trees to dominate in areas where summer is sufficiently long (Jarvis and Leverenz 1983). In contrast, conifers with winter resistant leaves are better adapted to regions with short summers as they do not have to renew their leaves each year and they can better utilize the marginal season (Waring and Franklin 1979; Aerts 1995). On the other hand, broad-leaved evergreen species are adapted to climates with mild winters, as there is no need to replace their leaves annually (Waring and Franklin 1979; Kira 1991).

In the case of roots, it is known that their phenological patterns are asynchronous to those observed in leaves, as reported for deciduous trees (Coté *et al.* 2003; Du and Fang 2014), grasses (Steinaker and Wilson 2008), shrubs and sedges in the arctic (Sloan *et al.* 2016). Although the pattern of leaf phenology has been well described (Thomas and Stoddart 1980; Buchanan-Wollaston 1997; Quirino *et al.* 2000; Lim *et al.* 2007), root phenology still remains understudied, given the challenges associated with extraction or assessment of the root system within its growing substrate (Rodgers *et al.* 2004; Yuan and Chen 2010; Lukac 2012). Interspecific variation in root turnover patterns is usually described along a continuous gradient based on their

root longevity (Ryser 1996). However, recent evidence shows that perennial monocotyledonous wetland species in high-latitude locations – Northern Ontario, Canada – fall in two categories, similar to deciduous and evergreen leaves: (1) species with annual roots which senesce in the autumn and, (2) species with overwintering roots which hardly experience any winter mortality and potentially last several years (Ryser and Kamminga 2009; Alsahame 2016; SJE Marcotte unpublished data). Roots of wetland species have mostly been studied for biomass production (Pilon *et al.* 2002; Lawrence *et al.* 2013), but no research has been done addressing their root overwintering strategy. As well, there is a lack of knowledge on the variation in root turnover within each strategy across different growing season lengths.

The observations by Ryser and Kamminga (2009) and Alsahame (2016) indicate a dichotomous root overwintering strategy in perennial wetland plants: species with autumn-senescing and species with overwintering roots. Species with annually renewed roots have traits such as fast growth rates (Gagnon 2014b) matching those of short-lived species known to be adapted to disturbances (Klimešová 2007). But to date, the adaptive advantages of the annually renewed roots are unknown as several of these species form stable stands in Ontario wetlands without an obvious disturbance (Harris *et al.* 1996; Coté 2017). The annual renewal of the entire root system is a constraining cost of their resource economics. Plants with autumn-senescing roots can be assumed to have delayed shoot growth in spring compared to plant species with overwintering roots, as new roots have to grow first (Gagnon 2014b). In Northern Ontario, in the Sudbury region, such species were observed to avoid the coldest soils, since the annual renewal of their entire root system requires either a long growing season or very good growth conditions to remain sustainable for the plant (Gagnon 2014a; Coté 2017).

Root senescence during an unfavourable season, such as dry season in arid regions, has been suggested to be advantageous due to high respiration costs of maintaining the roots during such season (Nobel *et al.* 1992). Whereas for overwintering roots in cold regions, the maintenance costs over the winter can be assumed to be minimal due to the low respiration rates in cool soils (Davidson 2006). Hendrick and Pregitzer (1993) reported consistently longer life spans for fine roots of sugar maple in a northern location compared to a warmer study location 80 km south. Roots of woody species were also observed to have a faster turnover rate with increasing soil temperature in the Mediterranean climate of California (Kitajima *et al.* 2010) and in the boreal forest (Yuan and Chen 2010). Analogous to overwintering roots, the needle leaves of evergreen coniferous trees have longer life spans as the climate gets colder and the growing season length decreases (Kudo *et al*. 1999). On the other hand, life span of deciduous leaves decreases with decreasing growing season length (Kudo 1992).

1.1 Research Objective

The objective of this research is to investigate to what extent the root dynamics of two contrasting types of root overwintering strategies – winter survival versus autumn-senescence – are affected by growing season length and winter soil temperatures. This project will investigate the species' phenological patterns with respect to root production and root senescence with a comparison of conspecific plants growing in contrasting climates. Specifically, this research will address the difference in timing of root senescence in the autumn and production in spring between a location with a long severe winter in Northern Ontario, Canada (46° 29' 42.8" N, 81° 00' 17.1" W) and another with a shorter warmer winter in North Carolina, USA (35° 18' 27.2" N, 81° 05' 24.3"). The present study will test the following hypotheses: (1) species with overwintering roots maintain the same strategy in both locations, but possibly with a slightly

higher winter survival rate in the northern location compared to the southern location due to higher soil temperatures in the latter, and (2) species with autumn-senescing roots maintain this strategy regardless of the winter's length, however their lifespans match the length of the growing seasons, i.e., in the southern location root lifespan is longer due to the longer growing season compared to the northern locations.

1.2 Significance

The proposed research has a two-fold significance. First, the seasonal pattern of leaf turnover of a species is known to be a reflection of adaptation to the local climate. Thus, knowledge on the relationship between climate and root seasonal patterns will potentially help to predict changes in relative abundance of wetland species resulting from the changing climate. Such changes in community composition have been documented as a response to the warming climate (Hillebrand *et al.* 2010; Keenan and Richardson 2015). Secondly, over half of the biomass production in northern wetlands is below ground, and understanding the relationships between seasonal root turnover patterns will help predict the effects of growing season length on the carbon and nutrient cycles (Lawrence *et al.* 2013; Finzi *et al.* 2015). Shi *et al.* (2015) observed that warming temperatures prolong the length of plant growing season and have a significant effect on nutrient dynamics within the plant delaying senescence and changing organ nutrient concentrations. It is well known that the seasonal pattern of leaf production and senescence of deciduous vegetation in the northern hemisphere results in seasonal fluctuations of the atmospheric CO_2 concentration (Keeling *et al.*1996; Strom *et al.* 2003; Kroger *et al.* 2007). Therefore, changes in abundances of species with contrasting root biomass turnover strategies may lead to changes in seasonal fluctuations of the atmospheric CO_2 concentrations.

2 Materials and Methods

2.1 Study locations

Two study locations were chosen based on their difference in growing season lengths (Table 1). Sudbury, ON, Canada, with an average growing season of 135 days, defined as the consecutive number of frost-free days (OMAFRA 2016), is located in the Great Lakes-St. Lawrence forest region towards the northern edge of the deciduous biome (Rowe 1972; Liu 1990). Gastonia, NC, USA, has an average growing season of 208 days (N. C. State Extension 1996), and is located towards the southern edge of the deciduous forest biome (Braun 1950; Dyer 2006).

In Sudbury, ON, the study location was an experimental garden (46° 35' 44.778" N, 81° 5' 49.42" W). The region is characterized by a humid continental climate with severe winters and warm summers (Dfb according to Köppen-Geiger Climate classification; Kotteck *et al.* 2006). The warmest month is July with an average maximum temperature of 24.8°C and the coldest month is January with an average minimum temperature of -17.9°C. The average annual precipitation is 903 mm and the average annual snowfall is 263.4 cm (Environment and Natural Resources Canada 2016a; Table 2 and 3). Greater Sudbury belongs to the Great Lakes-St. Lawrence Ecotonal Forest Region (Rowe 1972; Liu 1990).

In Gastonia, NC, the experimental site was in a wetland located in a protected portion of the Redlair Observatory known as the Spencer Mountain tract of the Catawba Land Conservancy (35° 18' 51.552" N, 81° 6' 4.97" W). Gastonia is located in the Piedmont physiographic province of North Carolina, 30 km west of Charlotte and to the southeast of the Appalachian Mountains, USA. The climate is characterized by short cool winters and hot humid summers (Cfa according to Köppen-Geiger Climate classification; Kotteck *et al.* 2006; State Climate Office of North

Carolina 2014b). The warmest month is July with an average maximum temperature of 31.9°C and the coldest is January with an average minimum temperature of -1.6°C. The average annual precipitation is 1091 mm and the average annual snowfall is 3 cm (US Climate data 2016; Table 2 and 3). In terms of forest regions, the location is in the southern edge of the Mesophytic Forest (Braun 1950; Dyer 2006). Edenton, NC (36° 03' 31.1" N, 76° 36' 28.7" W), origin of the plant material employed in the study (see below), is closer to the coast and the climate it is characterized by similar temperatures, but slightly higher precipitation compared the Redlair Observatory site. The warmest month is July with an average maximum temperature of 31.2°C and the coldest month is January with an average minimum temperature of 1.1°C. The average annual precipitation is 1220 mm and the average annual snowfall is 8 cm (US climate data 2016; Table 2 and 3).

2.2 Plant Material

Two monocot wetland species were used in the study: *Carex crinita* Lam. (Cyperaceae) known as fringed sedge and *Sagittaria latifolia* Willd. (Alismataceae) known as arrowhead or duck potato. Both species occur naturally in both of the study locations (NCU Flora of the Southeastern United States 2005; USDA, NRCS. 2006; Canadensys 2017; Personal observation). *C. crinita* is a freshwater graminoid that grows naturally along flood plains, riverbanks, swamps and bottom woodlands throughout the provinces of Manitoba to Nova Scotia except in Newfoundland and Labrador, Canada (Brouillet et al. 2010). In the USA it is found from Minnesota to Eastern Texas and extending to the eastern coast, except in the state of Florida (Godfrey and Wooten 1989; Marie-Victorin et al. 1995; USDA, NRCS 2006; Voss and Reznicek 2012). In eastern North America, Sudbury, ON lies at the northern edge of the continuous distribution of *C. crinita* (Canadensys 2016), while in the south the continuous distribution of the

species extends to South Carolina and northern counties of Georgia, USA (NCU Flora of Southeastern United States 2005; USDA NRCS 2006). This species is a perennial wetland plant with overwintering rhizome, roots and winter buds, which are green overwintering shoot tips with arrested growth. (Godfrey and Wooten 1989; Marie-Victorin et al. 1995; Voss and Reznicek 2012; personal observation).

S. latifolia is an emergent freshwater plant that grows naturally along river banks and shores of lakes and ponds throughout all the southern provinces from British Columbia to Nova Scotia, Canada (Brouillet *et al.* 2010). In the USA is found in all states except in Nevada (Godfrey and Wooten 1989; Marie-Victorin *et al.* 1995; Voss and Reznicek 2012). In eastern North America, Sudbury, ON is close to the northern edge of the continuous distribution (Canadensys 2016) of *S. latifolia*, while Gastonia, NC is close to the southern edge of the continuous distribution in the northern counties of South Carolina (NCU Flora of Southeastern United States 2005; USDA NRCS 2006). However, the species also occurs in Florida and in Central and South America (Flora of North America Editorial Committee, 1993+; Zepeda-Gómez and Lot 2005). This forb is classified as a perennial plant since it has vegetative overwintering structures known as corms (Godfrey and Wooten 1989; Marie-Victorin *et al.* 1995; Voss and Reznicek 2012). Nevertheless, it can be considered to be a pseudo-annual, i.e., functionally like an annual plant since all parts of the mother plant senesce and decompose before the winter season leaving only the propagules known as corms over the winter (Verburg and Grava 1998). In Vermilion River (46° 23' 52.758" N, 81° 18' 13.662" W) in the Sudbury District (Ontario), both species were observed to grow in close vicinity of each other near the river edge with *S. latifolia* closer to the flooded shoreline and *C. crinita* in a slightly higher location but still flooded in the spring.

In each of the two study locations, local plant material was used. In Sudbury, Ontario, fifty plants per species were obtained from wild populations. For *C. crinita*, a large tussock was extracted from a stream crossing the old highway 69 south of the city (46° 24' 22.758" N, 80° 52' 35.082" W) on 16 April 2016 and separated into winter buds with about 2 cm of roots each. *S. latifolia* was grown from corms obtained from a population in the Vermilion river crossing the Trans Canadian Highway 17 near Whitefish, ON (46° 24' 2.28" N, 81° 18' 57.99" W) in June 2012 and grown in subsequent years at the experimental garden. Fifty corms were harvested on 16 April 2016 for the present experiment. In Gastonia, NC, the plant material was obtained from a nursery where the plant material was grown from seeds collected from the local plant populations in the area (Wetland Plants Inc., Edenton, NC, USA). The seedlings were initially grown in plugs of 110 ml.

2.3 Growth Conditions

In Sudbury, the fifty replicates per species were initially grown in 300 ml cups until their transplantation into 10 L mesocosms on 13 June 2016. The growth a substrate consisted of sieved wetland topsoil from a former beaver meadow (pH 4) with 26% organic matter content (Bainbridge, construction, North Bay, ON). The mesocosms were placed in 5 pools of 2.5 m length by 0.85 m width and 30 cm deep in alternating rows of *C. crinita* and *S. latifolia* (Fig. 1a). The experimental pools were filled with ground water and the water levels fluctuated 3-5 cm in the warmest months.

In Gastonia, plugs of the fifty replicates per species were transplanted into 7.5 l mesocosms on 12 May (*C. crinita*) and 8 June 2016 (*S. latifolia*). The mesocosms were lined with Vigoro weed block diamond polyethylene fabric (Vigoro Inc., Sylacauga, AL, US) at the bottom to prevent root growth outside the container to the soil. The two species were

transplanted in a growth substrate consisting of 1.3 l of Premium play sand at the bottom of the mesocosms with pH 6.5 (Quikrete Inc., Columbus, OH, US, No. 1113), followed by 6.8 l of Timberland Topsoil with pH 5.7 (Oldcastle Lawn & Garden Inc., Atlanta, GA, US) and a top 4 cm layer of Vigoro marble chip rock (Vigoro Inc., Sylacauga, AL, US) to protect the topsoil from being washed away by water and to deter weed growth. The mesocosms with the plant material were placed in two trenches of 3.7 m × 1.2 m × 30 cm deep at 3 m (*C. crinita*) and 2 m (*S. latifolia*) from the creek shore. Both trenches were covered with chicken wire to protect against herbivores such as geese and ducks (Fig. 1b).

Water levels in the trenches fluctuated between the trench bottom and 2 cm above the edges of the mesocosms depending on precipitation, but the soil never dried out. Soil moisture within the mesocosms was at the lowest level on 27 June 2016 with 0.27 m^3m^{-3} and at the highest level on 18 March 2017 with 0.53 m^3m^{-3} measured by the Hobo Weather Station (Model U30 NRC, Onset Computer Corporation 2007, Bourne, MA, USA). Both trenches remained continuously flooded from November 2016 until May 2017 when the experiment was concluded.

2.4 Soil Characteristics

Substrate analyses included pH, particle size and organic carbon content (UNC Charlotte Geography & Earth Sciences Soils Laboratory Manual 2016) for the sand in Gastonia and the potting soil in both locations (Table 4). Additionally, the three types of substrates were sent for nutrient analysis (Total Kjedahl N and Ca, Mg, Fe, P, by BCSALM protocol; Testmark Laboratories, Sudbury, ON). The results reported that the growth substrates in both sites are comparable for all plant nutrients except for the TK-nitrogen, which was higher for the substrate in Sudbury (Appendix 1). In Gastonia, one sample of the surrounding soil per trench was taken at each harvest and sent for routine soil analysis for percent humic matter content, pH, cation

exchange capacity and nutrient content (Mehlich-3 Extraction) at the North Carolina Department of Agriculture and Consumer Service (NCDA & CS; Appendix 2). The sampling was done according to the protocol for soil sampling of large areas (NCDA&CS 2014). The results indicated a seasonal peak of P in the spring at harvest 4. In Gastonia the water of the creek next to the trenches was tested for Total Organic Carbon, Total Nitrogen and the ions Na, NH_4, K, Mg, Ca (Appendix 3). The results indicated no major addition of nutrients to the plant material. In Sudbury the plants stood in groundwater with no additional nutrients.

2.5 Soil and Air Temperatures

In Sudbury, ten iButton® temperature data loggers per trench (DS1921G-F5# Maxim Integrated, San Jose, USA) were placed in the substrate of the mesoscosms and evenly distributed throughout the 5 pools. In Gastonia, NC, four iButton® temperature data loggers per trench were placed inside the mesocosms and evenly distributed to record substrate temperature every 255 minutes (Appendix 4). Additionally, a HOBO Weather Station was installed between the two trenches to record air temperature and soil moisture in the mesocosms substrate and the natural substrate every 10 minutes.

At the experimental garden in Sudbury, ON the warmest air temperature was 33 °C recorded on 18 June 2016 and the coldest was -34.5 °C on 9 February 2017. At the experimental site in Gastonia, NC the warmest air temperature was 36.6 °C recorded on 26 July 2016, and the coldest was -13.1 °C on 8 January 2017. Based on the air temperatures recorded in each location, the growing season in 2016 was 131 days in Sudbury and 216 days in Gastonia. Both values were within one standard deviation of the long-term averages.

To compare the experimental location with natural growing conditions of the species in Sudbury, ten iButton temperature data loggers were placed at 20 cm depth next to growing plants

of *C. crinita* and *S. latifolia* along the shores of Vermilion River in Sudbury, ON (46° 24' 2.28" N, 81° 18' 57.99" W) from September 2016 until August 2017 (Appendix 5). In Gastonia two iButton temperature data loggers were placed in the natural trench substrate from 9 May 2017 to 28 January 2018 to a depth of 10 cm, similar to the depth at which the mesocosms were embedded in each trench. Both trenches were next to a natural community of *S. latifolia* (Appendix 6).

2.6 Plant Harvest

In both locations, four destructive harvests were conducted throughout a full year of plant growth by retrieving 10 mesocosms per species at each harvest. The harvests in the two locations were conducted at different calendar days but similar dates with respect to the progress of the growing season. Since the average growing season in Gastonia is 73 days longer than in Sudbury, by lasting about one month longer in the autumn, and beginning about one month earlier in spring; the autumn harvests were conducted about one month later and the spring harvests one month earlier in Gastonia compared to Sudbury (Table 5). The four harvests were conducted in both locations according to the stage of their respective events of the growing season: late growing season, end of the growing season, end of winter, and early growing season. The intervals between harvests as described above will be referred as autumn, winter and spring respectively.

The plant material was thoroughly washed and separated in shoots, roots, stolons and corms for *S. latifolia* and in shoots, roots and rhizomes for *C. crinita*. A representative one-quarter of the root system was sampled and cut in segments of about 1 cm long from which 1/3 was used for measurement of specific root length using the grid-intersection method (Newman 1966; Tennant 1975). The remaining 2/3 of roots sampled were immersed in 20 ml vials with a

solution of 0.5 % 2,3,5-triphenyltetrazolium-chloride (TTC) and 10 mMol glucose in tap water in Sudbury and deionized water in Charlotte and incubated at 30 °C for 48 hours. Living roots stained red after the reduction of TTC to formazan in metabolically active roots (Comas *et al.* 2000). The glucose helps to promote cell metabolism enhancing the red pigmentation of metabolically active roots (Robey 2007). Before incubation, the vials with the roots and TTC solution were exposed to a vacuum at 6.5 kPa in Sudbury and 22.4 kPa in Charlotte for 10 minutes. The purpose of the vacuum was to remove the air from the aerenchymatous roots and improve TTC penetration into the root tissue (Brunner *et al.* 2002). A control for the TTC effect on root colour was prepared by heating a root sample to 70°C for 10 minutes and then treating it with TTC (Fig. 2). The stained roots were analyzed under the microscope to count the number of living and dead basal (>400 μm) and fine lateral (<400 μm) roots. Living roots were identified as having a red pigmentation resulting from TTC reduction to formazan in metabolically active roots while roots showing no change in colour were deemed as dead (Comas *et al.* 2000; Robey 2007; Ryser and Kamminga 2009). Dry masses were obtained after drying the plant material at 75 to 80°C for a minimum of 48 hours.

2.7 Calculated Variables

Specific root length (SRL) of the measured sample was calculated as length per dry mass. The total root length (TRL) per plant was calculated based on SRL and total root mass. The root length alive per plant was obtained by multiplying the total root length with the percentage of living roots. The dead root length was calculated as the difference between the total and living root length.

Growing degree-days (GDD) refers to the accumulation of daily heat above a threshold temperature for a plant to reach maturity (Danneberger and Cushnahan 2004; Agriculture and

Agri-Food Canada 2017). For this experiment, GDD were calculated with a base temperature of 0ºC using the recorded temperatures in the substrate of the mesocosms. The calculated growing degree-days are presented for each month during the study periods and for each interval between harvests. Growing degree-days were also calculated for the field-sites of *C. crinita* and *S. latifolia* along Vermilion River in Sudbury, ON (Appendix 5) and in Gastonia (Appendix 6) to compare with those obtained in the mesocosms.

2.8 Data Analyses

The analyzed variables include dry mass of shoots, roots, rhizomes (*C. crinita* only), stolons and corms (*S. latifolia* only), total root length, specific root length, percent roots alive and root length alive. Calculations were obtained using Microsoft Excel (Version 2011, Microsoft Inc.) and the data analysis was completed using SYSTAT 12.1 (2009 Systat Software, San Jose, CA). The data were tested for normality with a K-S test. Transformations were applied on the following variables to achieve normal distribution. Arc sin transformation was used for shoot dry mass and square root transformation for rhizome and root dry masses of C. crinita. The remaining variables of C. crinita (specific root length, percent living roots, living and total root length) were log transformed. For S. latifolia, a log transformation was used on the shoot, stolon and root dry masses and living and total root lengths. A total of 4 outliers were removed from the data analysis. For *C. crinita* two plants were removed from the Sudbury data in harvest 3, one with only a flowering shoot while all other plants had flowering and vegetative shoots, and another plant which was partially eaten during the winter by voles. For *S. latifolia*, one case in each, the living and total root length from Gastonia data in harvest one were removed from the analysis based on a studentized residual above 4. The two outliers belonging to different

replicates were removed from the above-mentioned variables due to possible loss of root mass during the cleaning process and resulted in measurements much lower than the average.

General linear models were used to test the effects of harvest, location and the interaction on the measured traits in *C. crinita* and for the corms of *S. latifolia*. Post-hoc analysis was followed with a Tukey's difference test. Except for the corms, there was no other living plant material for *S. latifolia* in Sudbury in harvest 3, only harvests 1 and 2 were used to test the effects of harvest, location and their interaction on the measured variables. Data of harvest 4 referring to the new plant material in *S. latifolia* was tested separately with a general linear model using only location as the independent factor. Additionally, plants of *S. latifolia* in Gastonia maintained some roots and stolons from the growing season 2016 throughout the four harvests. These variables were tested with a general linear model using only harvest as a factor. In the case of the percentage of living roots for *S. latifolia,* data could not be normalized and a non-parametric Kruskal-Wallis test was used separately for harvest 1 and 4 between locations. There was no variation in the data of harvest 2 and 3 in Sudbury (100% mortality). An additional Kruskal-Wallis test for Gastonia was used to test harvest effects on percentage root survival since a portion of the roots remained alive throughout the four harvests.

Table 1. Summary description for the two study locations Sudbury, ON and Gastonia, NC. The first and last frost dates are defined as the first or last day with an air temperature of 0 °C (OMAFRA 2016, U. S. climate data 2016 and N.C. State extension 1996). The growing season for 2016, the dates of first and last frosts were calculated based on the air temperatures taken at each location.

Characteristic / Site location	Sudbury Ontario, Canada	Gastonia North Carolina, USA
Geographical coordinates	46° 35' 44.778"N 81° 5' 49.42"W	35° 18' 51.552"N 81° 6' 4.97"W
Avg. growing season length	135 ± 10 days*	208 ± 22 days*
Growing season 2016	131 days	216 days
Avg. date of first fall frost	26 Sep ± 14 days*	1 Nov ± 14 days*
Date of first fall frost (2016)	24 Sep 2016	11 Nov 2016
Avg. date of last spring frost	17 May ± 14 days*	6 Apr ± 14 days*
Date of last spring frost (2017)	20 May 2017	23 Mar 2017

* ± 1 standard deviation (SD)

Table 2. Monthly long-term average minimum and maximum temperatures, precipitation and snow depth for Sudbury, Ontario from 1981 – 2010 (Environment & Natural Resources Canada 2016a), Gastonia and Edenton, North Carolina from 1971 – 2000 (U.S climate data 2017, State Climate Office of North Carolina 2014a).

Month	Sudbury, ON				Gastonia, NC				Edenton, NC			
	Avg. Max ° C	Avg. Min ° C	Avg. Precip. mm	Avg. snow depth cm	Avg. Max ° C	Avg. Min ° C	Avg. Precip. mm	Avg. snow depth cm	Avg. Max ° C	Avg. Min ° C	Avg. Precip. mm	Avg. snow depth cm
January	-8	-17.9	62.2	30	10.6	-1.6	106.9	2.5	10.6	1.1	105.7	2.5
February	-5.5	-16	51.1	38	13.1	0.1	95.3	0	12.6	2.1	84.3	2.5
March	0.4	-10.2	60.5	27	17.5	3.8	108.2	0	16.9	5.3	109.5	0
April	9.2	-1.7	65.7	2	22.3	8.7	73.4	0	21.7	9.9	82.3	0
May	17	5.2	83.4	0	26.4	13.8	92.5	0	25.8	14.8	107.4	0
June	22.2	10.7	80.3	0	30.3	18.7	93.5	0	29.7	19.8	113.3	0
July	24.8	13.4	76.9	0	31.9	20.8	89.7	0	31.2	22	131.3	0
August	23.4	12.4	85.4	0	31.2	20.3	103.9	0	29.9	21.2	134.6	0
September	18.1	7.8	101.1	0	27.8	16.5	108.2	0	26.9	17.9	119.1	0
October	10.3	1.7	90.9	0	22.6	9.7	95.5	0	22.2	11.8	92.5	0
November	2.6	-4.7	78.5	3	17.4	4.4	80.3	0	17.2	7.1	71.4	0
December	-4.4	-12.7	67.5	16	12.1	0.1	90.7	0	12.2	2.7	76.7	2.5

Table 3. Monthly average minimum and maximum temperatures, precipitation and snow depth for Sudbury, Ontario (Environment and Natural Resources Canada 2016b) and Gastonia, North Carolina (U.S. climate data 2016) during the current experiment.

Month	Sudbury, ON				Gastonia, NC			
	Avg. Max. ° C	Avg. Min. ° C	Avg. Precip. mm	Avg. snow depth cm	Avg. Max. ° C	Avg. Min. ° C	Avg. Precip. mm	Avg. snow depth cm
				2016				
May	19.1	5.4	53.4	0	25.7	13.8	145.7	0
June	23.7	9.4	36.1	0	32.3	19.4	88.6	0
July	25.9	13.3	81.8	0	33.8	21.7	70.3	0
August	25.6	14.3	171.1	0	33.8	22.4	74.7	0
September	20.7	10.0	68.4	0	31.5	19.3	35.6	0
October	12.0	2.7	95.6	0	25.2	11.9	61.0	0
November	6.4	-1.5	51.4	2	19.8	5.2	11.9	0
December	-4.0	-11.6	76.5	22	13.1	3.1	67.9	0
				2017				
January	-3.9	-11.2	51.1	38	13.6	2.9	137.2	0.2
February	-2.8	-12.2	78.9	36	18.6	4.2	27.7	0
March	-1.6	-12.2	56.6	19	18.4	3.8	61.0	0.1
April	9.1	-0.7	138.4	2	23.9	12.2	140.1	0
May	15.6	4.8	99.0	0	26.4	14.5	186.7	0
June	21.2	10.6	113.3	0	29.7	19.0	84.9	0

Table 4. Summary results for soil analysis for pH, % Organic carbon content (OCC), particle size and shape for on the playsand and topsoil used in Gastonia and the wetland soil in Sudbury. Measurements based on UNC Charlotte Geography & Earth Sciences Soils Laboratory Manual (2016).

Soil type	pH	% Weight OCC	Particle Size	Particle shape
Play sand	6.5	19.2	700-1000 250-350	Angular sandy crystals
Topsoil (Timberland)	5.7	0.444	2-3 mm 500-700 μm	Subangular and subrounded organic particles Angular sandy crystals
Topsoil (Bainbridge)	4	35.5	1.4-2 mm 88-250 μm 500-1000 μm	Subangular and subrounded organic aggregates Subrounded and rounded organic particles Angular sandy crystals

Table 5. Harvesting schedule for Sudbury, ON and Gastonia, NC throughout a year of plant growth for *S. latifolia* and *C. crinita* from June 2016 to June 2017. The dates of the harvests differed between sites, but matched the different stages of plant growth.

Site/ Harvest	1	2	3	4
Sudbury, ON	26 Aug 2016	21 Oct 2016	23 Mar 2017	02 June 2017
Gastonia, NC	18 Sep 2016	14 Nov 2016	17 Feb 2017	01 May 2017
Growing season	Late GS	End GS	End Winter	Early GS
Season	Autumn	Winter	Spring	

a) b)

Figure 1. a) Experimental Garden in Sudbury, ON with 5 experimental pools containing alternating diagonal rows of both plant species. b) Experimental setup in Redlair Natural Preserve, Gastonia, NC with two trenches, *C. crinita* at the back and *S. latifolia* at the front of the picture.

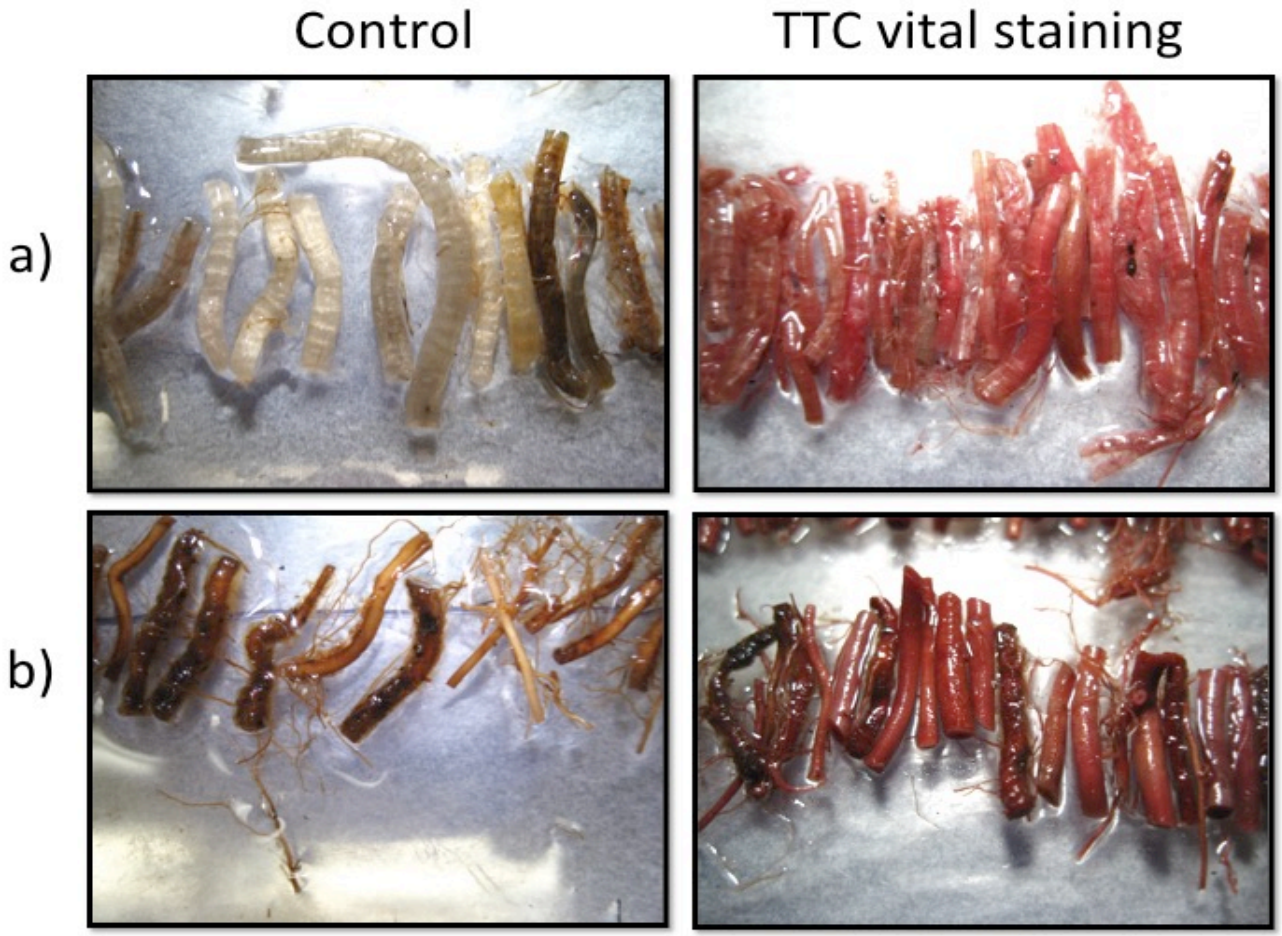

Figure 2. Roots of a) *S. latifolia* and b) *C. crinita* after TTC vital staining. The controls consisted of roots heated to 70°C for 15 min prior to TTC staining.

3 Results

3.1 Temperature At Study Sites

Temperatures in the growth substrate in Gastonia were warmer than in Sudbury by 6-8°C from May to September 2016, by 7-9°C from October 2016 to February 2017 and by 10-13°C from March to June 2017 (Table 6). In Sudbury, the mesocosms of both species were alternating in the same pools with no difference between the substrate temperatures of *C. crinita* and *S. latifolia*. However, in Gastonia, the two species grew in separate trenches with a slight difference in water level due to proximity to the creek, there was a small temperature difference of up to about 1°C. The *S. latifolia* trench was warmer than the *C. crinita* trench in summer and winter, but cooler in autumn and spring.

The heat sum between locations differed considerably. Plants in Gastonia received about twice as much heat in the summer and spring, compared to plants in Sudbury. During the autumn the heat sum varied less between the two locations with 20-30% higher growing degree-days (GDD) in Gastonia. During the first summer in Gastonia, the three-week difference in planting dates resulted in 15% higher heat sum for *C. crinita* mesocosms compared to *S. latifolia* before the first harvest. During the winter, Sudbury plants received half of the heat accumulated by plants in Gastonia and with no difference between the two trenches (Table 7).

The temperatures between the mesocosms and the field substrates differed by 1-2°C, displaying higher fluctuations inside the mesocosms than in the field at both locations. However, in the field and mesocosms there were no temperatures registered below 0.9°C in Sudbury and 2°C in Gastonia. In Sudbury and Gastonia the heat sums between the mesocosms and the natural field substrate differed seasonally. In autumn, the substrate in the mesocosms had 40% lower GDD compared to the natural substrate in the field, however in winter the trend was inversed

with 50% higher GDD for the mesocosms than the field. In spring the field sites had 30% higher GDD values than the mesocosms (Appendix 5). In Gastonia, the mesocosms in both trenches had an average 15% higher heat sums during the summer compared to the natural substrate and an opposite trend from middle autumn and winter. In autumn both trenches had about 30% lower GDD values compared to the natural field substrate (Appendix 6).

3.2 *Carex Crinita:* Overwintering Root Strategy

In Sudbury, some replicates of this species flowered. No significant differences were found between plants with only vegetative shoots and plants with vegetative shoots and flowering stems for any of the traits measured throughout the experiment. Nevertheless, plants with flowering shoots tended to be smaller with a mean 30% lower for the aboveground dry mass (p= 0.061; Appendices 7 and 8). In Sudbury, five plants of each category were sampled every harvest, with an exception of harvest 3 where two plants with vegetative shoots and seven plants with vegetative and flowering shoots were collected. The unbalanced plant collection in the third harvest was due to difficulties in obtaining the planned replicate mesocosms under a thick layer of snow and ice covering the straw and tarps that protected the plant material. This may have resulted in plants being slightly smaller than they would have been if the collection could have been done as planned.

3.2.1 Aboveground traits

The seasonal pattern of leaf phenology in *C. crinita* was similar in both locations with green shoots in summer and autumn, only winter buds during the winter and fully developed leaves in spring. The shoot dry mass showed no significant changes during the autumn in both locations. During the winter, shoot dry mass decreased significantly in both locations, consisting

in harvest 3 only of the green overwintering shoot tips with arrested growth. In spring the shoots resumed growth. Shoot dry mass of *C. crinita* was 30% higher before the winter in Sudbury compared to Gastonia, however after the winter and in harvest 3 and 4 the shoot dry mass in Gastonia was 30% and 25% larger, respectively (Fig. 3a-b; Appendix 9). This resulted in a significant harvest × location interaction for the shoot dry mass (Table 8; Fig. 10), in spite of the similarity for the overall phonological pattern in both locations.

3.2.2 Belowground traits

The rhizome dry mass increased in autumn with no further growth during the winter and spring (Fig. 3c-d) with no significant general difference between plants in Sudbury and in Gastonia. Nevertheless, harvest × location interaction was weakly significant due to an 80% increase of the rhizome dry mass in Sudbury in autumn while in Gastonia the increase was only by 50% (Table 8; Appendix 9).

Root dry mass of *C. crinita* was on average 35% higher in Sudbury plants compared to Gastonia ones throughout the four harvests. In both locations the root dry mass showed a significant increase by more than 50% from harvest 1 to 2. During the winter the root dry mass decreased slightly but not significantly. The highest root dry mass values were observed in harvest 4 at both locations (Fig. 3e-f and 10; Appendix 9).

The specific root length was higher for Gastonia plants compared to Sudbury ones, with the exception of harvest 4 in which Sudbury plants had 15% higher values (Fig. 4 a-b). A significant harvest × location interaction effect was driven by the steady decrease of specific root length in Gastonia plants from harvest 1 to 4, while in Sudbury the decrease from harvest 1 to 3 was followed by an increase from harvest 3 to 4.

In Gastonia root survival, as indicated by the TTC staining, remained above 90% of the total root length throughout the seasons. In Sudbury the percentage of red-stained roots increased from 75% in harvest 1 to 86% in harvest 3 at the end of the winter. In the spring, the proportion of red-stained roots in both locations was above 95% (Fig. 4c-d; Appendix 9). The significant harvest × location interaction was driven by the increase in percent root survival in Sudbury plants from harvest 1 to 4.

The total root length per plant differed significantly among harvests with a similar seasonal variation pattern in both locations (Fig. 5a-b; Appendix 9). The seasonal fluctuations of the total root length were larger for Sudbury plants with about 80% increase in autumn and spring and 45% decrease in winter. For Gastonia plants, the root length increased by 30% in autumn but decreased by 30% in winter and remained the same in spring. Nevertheless, there was no significant harvest × location interaction, possibly due to the high within-harvest variation observed in Sudbury plants (Table 8).

There was a significant harvest effect on the root length alive, but no location effect. Harvest × location interaction was close to significance (p=0.076), resulting from the large fluctuations among the harvests in Sudbury plants (Table 8). The root length alive increased from harvest 1 to 2 by 45% and 25% in Sudbury and Gastonia plants respectively, but in winter Sudbury decreased by 40% and Gastonia by 30%. In the spring the root length alive increased again by 50% in Sudbury but remained the same in Gastonia (Fig. 5c-d; Appendix 9).

3.3 *Sagittaria Latifolia:* Autumn-Senescing Root Strategy

3.3.1 Aboveground traits

There was a significant difference between Sudbury and Gastonia plants in the variation of shoot seasonal patterns before the winter with a significant harvest × location interaction

(Table 9). In harvest 1 both locations had similar shoot dry mass, but in harvest 2, shoots in Sudbury were mostly senesced while the Gastonia plants still had few green leaves with about a quarter of the shoot biomass observed in harvest 1. At the end of the winter there was no shoot mass left in either location. In harvest 4 new leaves emerged with no significant difference in shoot mass between locations (Table 9; Figs. 6a-b and 11).

3.3.2 Belowground traits

Both stolon and root masses strongly decreased throughout the harvests in both locations but with different speed and extent (Figs. 6c-f and 11). Before the winter, the stolon dry mass was 35% higher in Sudbury plants compared to Gastonia ones and decreased in harvest 2 by 75% in Sudbury and by 55% in Gastonia (Table 9). There was a significant but weak harvest × location interaction effect on the stolon dry mass (harvests 1 and 2; $p=0.036$). After the winter in harvest 3, there were no stolons in Sudbury plants, while for Gastonia plants 15% of the stolon dry mass recorded in harvest 1 was still present. In the last two harvests in Gastonia, few of these stolons were included with the roots treated with TTC, and they stained red being obviously alive. In three plants the stolons held a connection between the corms and the root system grown in 2016. Some of the stolons remained alive until harvest 4 and in one plant the stolons connected the remaining alive roots grown in 2016 with a new emerging plant with the corm in between. In spring, the new stolons began growth with no significant difference in dry mass between locations (Fig. 6c-d; Appendix 10).

In early autumn, plants in both locations had similar root dry mass, but by harvest 2 in Sudbury the root dry mass decreased sharply by 75%, while in Gastonia the decrease was only by 10%. After the winter in harvest 3, no root dry mass was present in Sudbury plants whereas in Gastonia half of the root dry mass recorded in harvest 1 was still present. In harvest 4, a quarter

of the root dry mass produced in the previous growing season and recorded in harvest 1 could still be washed out of the mesocosms. Additionally, by harvest 4, plants in both locations began growing new roots with a significant difference of 40% higher root dry mass in Sudbury plants compared to Gastonia ones (Fig. 6e-f; Appendix 10).

In harvest 1, the specific root length was 22% higher in Sudbury plants than in Gastonia ones. However, the high mortality observed in harvest 1 and 2 did not provided any conclusive information for the specific root length and it was not further analyzed. The specific root length of the new cohort of roots grown in 2017 was 40% higher for Sudbury plants (Fig. 7a-b; Appendix 10).

In harvest 1, only 30% of the root length of Sudbury plants stained red, while 50% root system stained red for Gastonia plants. By harvest 2, all roots were dead in Sudbury plants, while in Gastonia, some roots survived through the winter into the spring (Fig. 7c-d). Due to decomposition of dead roots any percentage values were meaningless to indicate survival, but were used to calculate the length of the living root length. In harvest 4, the new cohort of roots emerged being 100% alive in both locations (Table 10; Appendix 10).

The total root length showed a similar pattern as that observed on the root dry mass. For the first two harvests, there was a significant harvest × location interaction effect driven by the sharp decrease in Sudbury plants, opposed to the gradual decrease in Gastonia plants (Table 9). The total root length of the new cohort of roots grown by harvest 4 was 60% larger for Sudbury plants (Fig. 8a-b; Appendix 10).

For harvest 1 and 2 the total root length alive, as determined by TTC staining, differed significantly between harvests, locations and had a significant harvest × location interaction (Table 10). The values were similar in both locations in harvest 1, but by harvest 2 the root

length alive decreased in Sudbury plants by 99%, whereas in Gastonia the decrease was by 85%. After the winter no living roots were found for Sudbury plants, while for Gastonia plants some roots survived until spring, with living root lengths of 5-15% of the values recorded in harvest 1. The root length alive of the new cohort of roots grown in 2017 was 60% higher for Sudbury plants compared to Gastonia ones (Fig. 8c-d; Appendix 10). The dead root length had similar pattern as that observed on the total root length with a sharp decrease in Sudbury plants by harvest 2 but a gradual decrease in Gastonia plants from harvest 1 to 4, with a significant harvest × location interaction (Table 9; Fig. 8e-f).

In harvest 1, the mesocosms in Sudbury had about a ten-fold number of corms compared to Gastonia ones. The number of corms increased in both locations from harvest 1 to harvest 2, and in Gastonia further until harvest 3, reaching about a quarter of the number of corms recorded in Sudbury plants. In Gastonia, three plants in harvest 1 and one plant in harvest 3 had no corms at all and were not included in the calculation for average corm dry mass. In Gastonia, the corms had a considerably larger mass compared to Sudbury ones, with a difference of about 70% (Appendix 10). However, this difference was not significant due to the high variation in corm dry mass. The corm size and corm mass per mesocosm decreased by harvest 4 (Table 9; Fig. 9a-f).

Table 6. Average temperature and cumulative sum of growing degree-days (GDD) for the growth substrate of the mesocosms in each month during the study period from May 2016 to June 2017 at the two study locations. In Sudbury the values are averages of 10 data loggers distributed in five pools and in Gastonia they are the averages of 4 data loggers in each trench.

Site	Sudbury, ON		Gastonia, NC			
Species	*C. crinita* & *S. latifolia*		*C. crinita*		*S. latifolia*	
Month	Temperature (°C)	Monthly GDD	Temperature (°C)	Monthly GDD	Temperature (°C)	Monthly GDD
			2016			
May	15.9	393	19.9	388	20.2	398
June	18.2	499	24.1	720	25.3	756
July	20.8	594	26.2	813	27.6	854
August	20.1	637	25.7	798	26.8	839
September	15.3	472	24.0	721	23.2	699
October	9.1	26	17.8	550	17.2	512
November	4.5	174	10.6	318	11.4	346
December	2.5	98	7.6	236	8.5	266
			2017			
January	2.0	79	7.9	346	8.6	267
February	1.6	56	11.9	348	11.2	320
March	1.7	53	14.3	352	12.8	396
April	4.3	130	17.8	533	17.9	530
May	11.1	293	21.30	739	21.6	752
June	12.5	123	24.22	725	25.7	769

Table 7. Cumulative sum of growing degree-days (GDD) per harvest interval for *C. crinita* and *S. latifolia* in Sudbury, ON and Gastonia, NC throughout a year of plant growth from the experimental set up until the fourth harvest. The difference of cumulative GDD between *C. crinita* and *S. latifolia* in Gastonia is due to one-month difference between dates of the experimental set up. For dates of the harvests see Table 5.

Year	Harvest interval	Season	GDD
	Sudbury, ON – *C. crinita* and *S. latifolia*		
2016	Set up to harvest 1	Summer	1504
2016	Harvest 1 to 2	Autumn	811
2016-2017	Harvest 2 to 3	Winter	492
2017	Harvest 3 to 4	Spring	552
	Gastonia, NC – *C. crinita*		
2016	Set up to harvest 1	Summer	3124
2016	Harvest 1 to 2	Autumn	1042
2016-2017	Harvest 2 to 3	Winter	1085
2017	Harvest 3 to 4	Spring	1142
	Gastonia, NC – *S. latifolia*		
2016	Set up to harvest 1	Summer	2625
2016	Harvest 1 to 2	Autumn	983
2016-2017	Harvest 2 to 3	Winter	1085
2017	Harvest 3 to 4	Spring	1087

Table 8. Summary results of the general linear model (GLM) for traits measured in *C. crinita* with harvest and location as independent factors.

Dependent factor	Independent factors	*n*	R^2	F-ratio	*P*-value
Shoot dry mass	Harvest	78	0.716	54.5	<0.001***
	Location			0.02	0.881
	Harvest × loc.			4.6	0.006**
Rhizome dry mass	Harvest	78	0.531	23.5	<0.001***
	Location			0.1	0.725
	Harvest × loc.			2.9	0.040*
Root dry mass	Harvest	78	0.492	16.9	<0.001***
	Location			14.3	<0.001***
	Harvest × loc.			0.8	0.506
Total root length	Harvest	78	0.238	4.8	0.004**
	Location			1.8	0.184
	Harvest × loc.			1.9	0.137
% Roots alive	Harvest	78	0.881	55.1	<0.001***
	Location			2221.2	<0.001***
	Harvest × loc.			38.1	<0.001***
Alive root length	Harvest	78	0.255	5.7	0.002**
	Location			0.2	0.632
	Harvest × loc.			2.4	0.076
Dead root length	Harvest	78	0.661	17.9	<0.001***
	Location			65.9	<0.001***
	Harvest × loc.			4.6	0.005**
Specific root length	Harvest	78	0.649	32.2	<0.001***
	Location			14.1	<0.001***
	Harvest × loc.			6.3	0.001**

Table 9. Summary results of the general linear model for traits measured in *S. latifolia*, with harvest and location as independent factors.

Dependent factor	Independent factor	*n*	R^2	F-ratio	*P*-value
Shoot dry mass H1 & H2	Harvest	40	0.890	173.8	<0.001***
	Location			54.82	<0.001***
	Harvest × loc.			63.47	<0.001***
Shoot dry mass - H4	Location	20	0.088	1.733	0.205
Stolon dry mass H1 & H2	Harvest	40	0.696	74.17	<0.001***
	Location			3.709	0.062
	Harvest × loc.			4.731	0.036*
Stolon dry mass Gastonia	Harvest (1-3)	30	0.695	29.67	<0.001***
Stolon dry mass - H4	Location	20	0.000	0.000	0.989
Root dry mass H1 & H2	Harvest	40	0.547	20.28	<0.001***
	Location			10.11	0.003**
	Harvest × loc.			13.06	0.001**
Root dry mass Gastonia	Harvest (1-4)	40	0.364	6.877	0.001**
Root dry mass - H4	Location	20	0.259	6.304	0.022*
Total root length H1 & H2	Harvest	39	0.466	22.96	<0.001***
	Location			0.077	0.783
	Harvest × loc.			6.805	0.013*
Total root length Gastonia	Harvest (1-4)	39	0.488	11.14	<0.001***
Total root length - H4	Location	20	0.484	16.86	0.001**
Root length alive H1 & H2	Harvest	39	0.835	122.4	<0.001***
	Location			29.65	<0.001***
	Harvest × loc.			26.35	<0.001***
Root length alive Gastonia	Harvest (1-4)	39	0.399	7.512	0.001**
Root length alive - H4	Location	20	0.484	16.86	0.001**

To be continued

Table 9. Continuation

Dependent factor	Independent factor	*n*	R^2	F-ratio	*P*-value
Dead root length H1 & H2	Harvest	39	0.350	5.544	0.024*
	Location			0.642	0.428
	Harvest × loc.			12.25	0.001**
Dead root length Gastonia	Harvest (1-4)	39	0.637	20.45	<0.001***
Specific root length H1 & H2	Harvest	40	0.512	1.821	0.186
	Location			28.68	<0.001***
	Harvest × loc.			5.243	0.028*
Specific root length Gastonia	Harvest (1-4)	40	0.397	7.682	<0.001***
Specific root length - H4	Location	20	0.403	12.16	0.003**
Total corm dry mass per pot	Harvest	80	0.395	12.32	<0.001***
	Location			0.463	0.498
	Harvest × loc.			3.208	0.028*
Avg. corm dry mass per pot	Harvest	76	0.544	7.684	<0.001***
	Location			42.45	<0.001***
	Harvest × loc.			5.244	0.003**
Total number of corms per pot	Harvest	80	0.787	4.090	0.010*
	Location			247.8	<0.001***
	Harvest × loc.			2.167	0.099

Table 10. Summary results for Kruskal-Wallis tests of percent roots alive in *S. latifolia* with harvest and location as independent factors.

Dependent factor	Independent factor	*n*	Kruskal-Wallis	χ^2	d.f.	*p*-value
% Roots alive – Harvest 1	Location	20	21.0	4.8	1	0.028*
% Roots alive – Harvest 4	Location	20	50.0	0.0	1	1.000
% Roots alive Gastonia	Harvest (1-4)	40	21.4	.	3	<0.001***

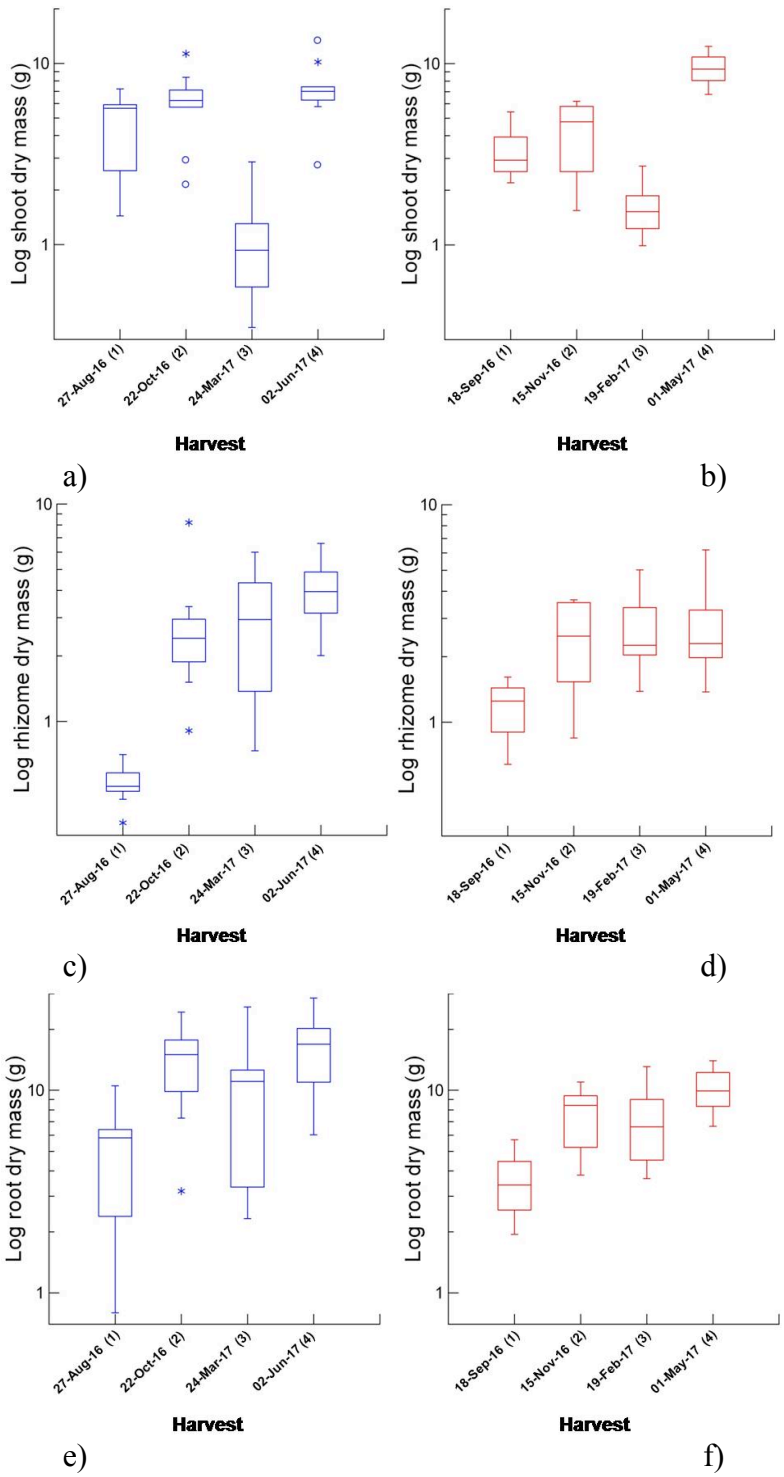

Figure 3. Boxplots for shoot, rhizome and root dry masses of *C. crinita* in the four harvests in Sudbury, ON (a, c, e) and Gastonia, NC (b, d, f).

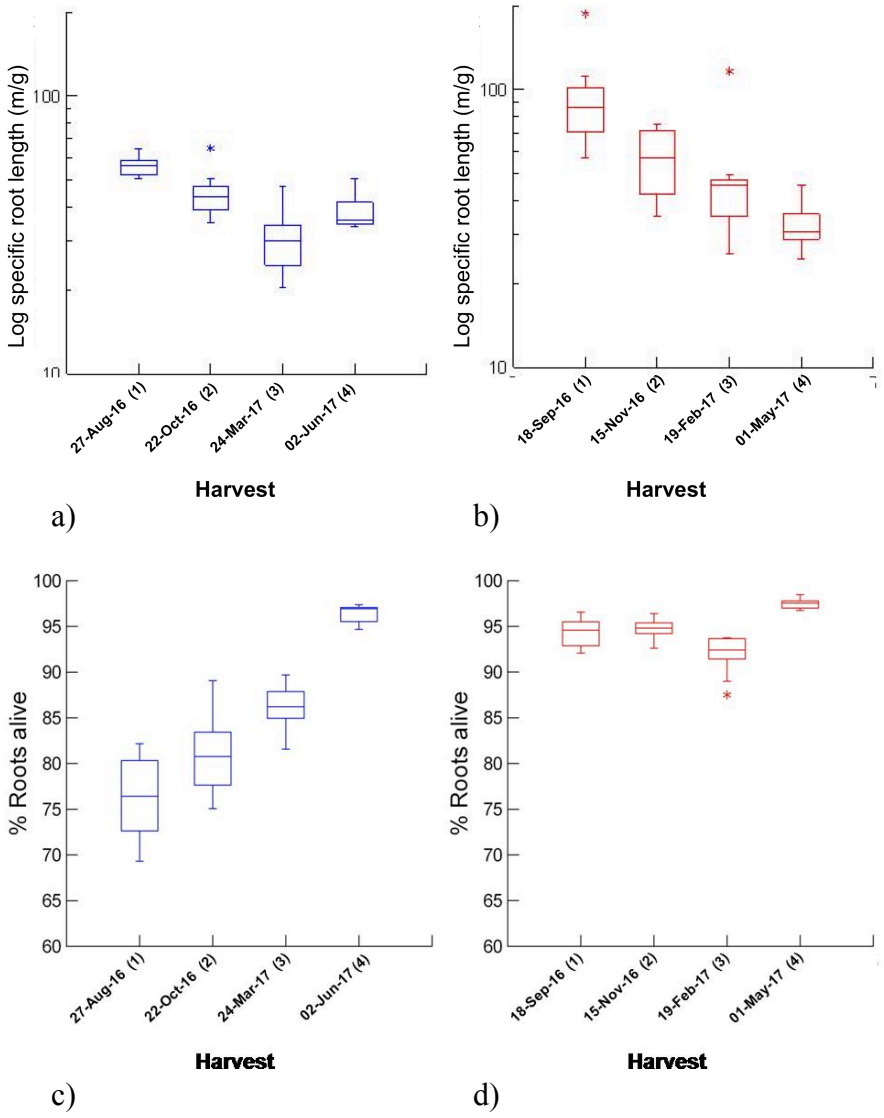

Figure 4. Boxplots for specific root length and percent roots alive of *C. crinita* in the four harvests in Sudbury, ON (a, c) and Gastonia, NC (b, d).

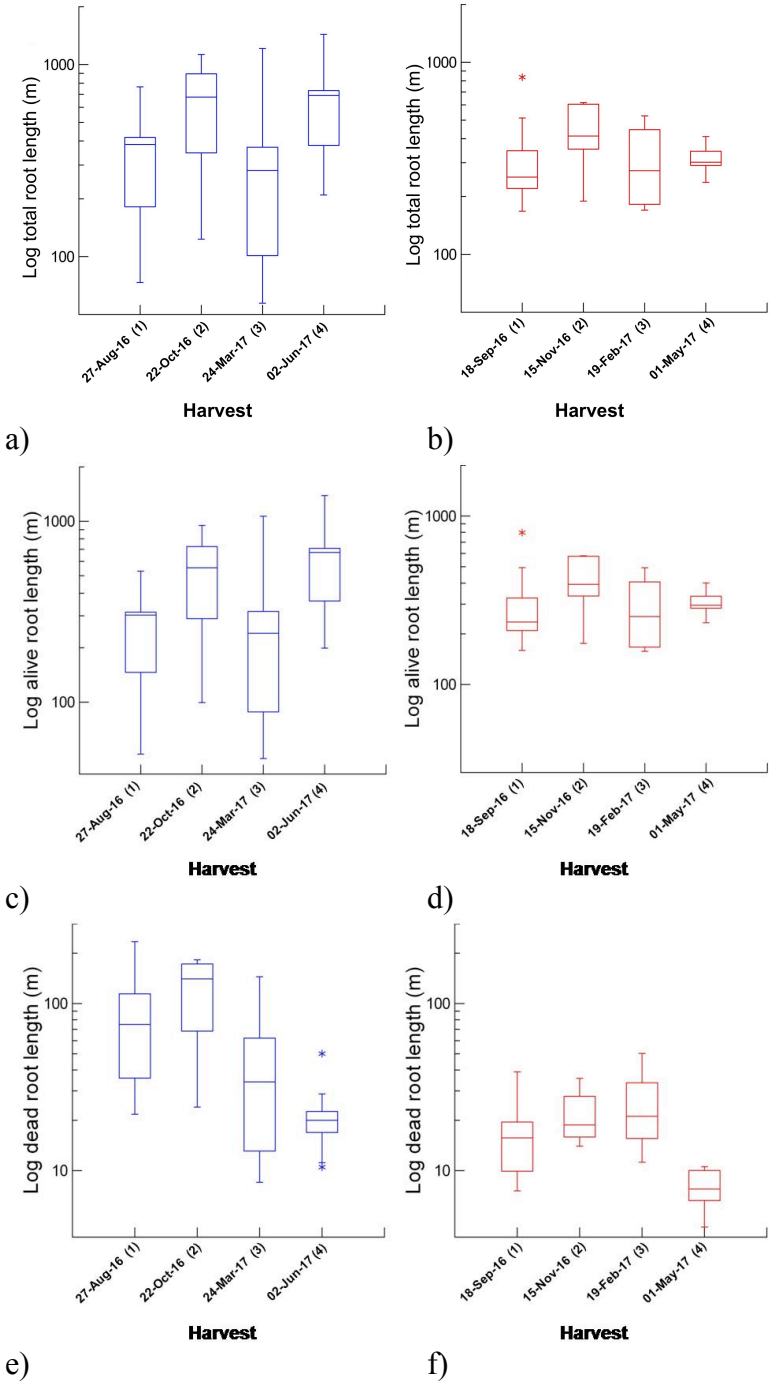

Figure 5. Boxplots for total, alive and dead root length of *C. crinita* in the four harvests in Sudbury, ON (a, c, e) and Gastonia, NC (b, d, f).

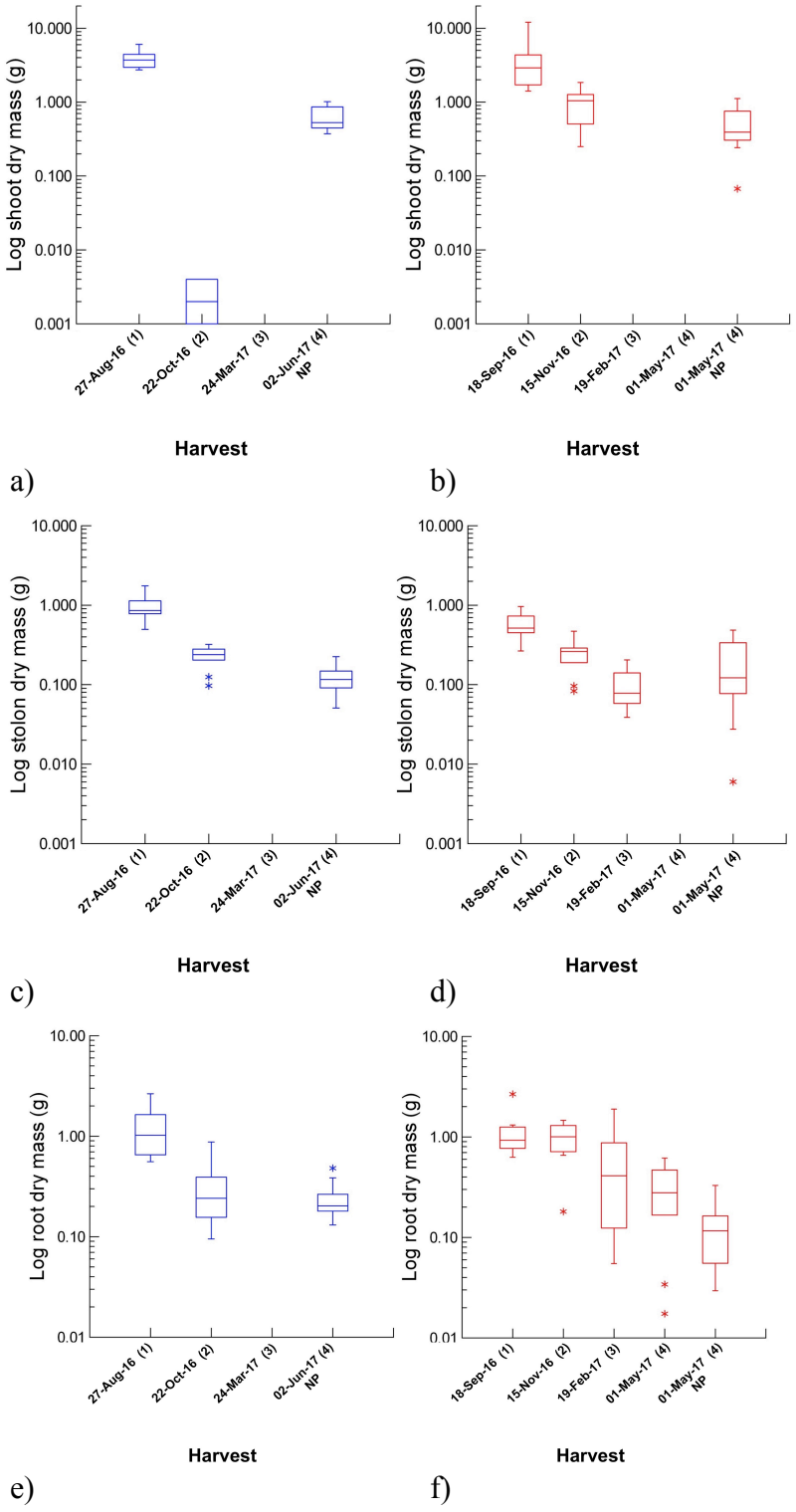

Figure 6. Boxplots for shoot, stolon and root dry masses of *S.* Boxplots for shoot, stolon and root dry masses of *S. latifolia* in the four harvests in Sudbury, ON (a, c, e) and Gastonia, NC (b, d, f) where NP stands for the new plant material grown in spring of 2017.

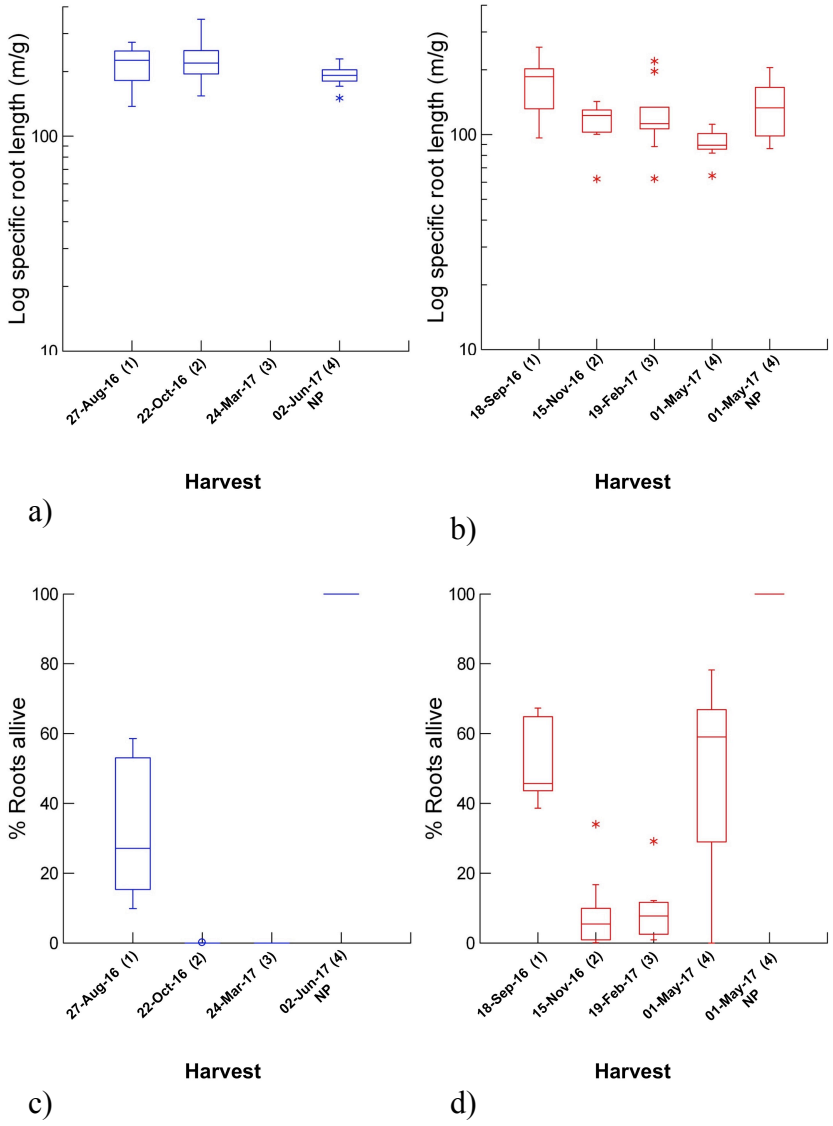

Figure 7. Boxplots for specific root length and percent roots alive of *S. latifolia* in the four harvests in Sudbury, ON (a, c) and Gastonia, NC (b, d) where NP stands for the new plant material grown in spring of 2017.

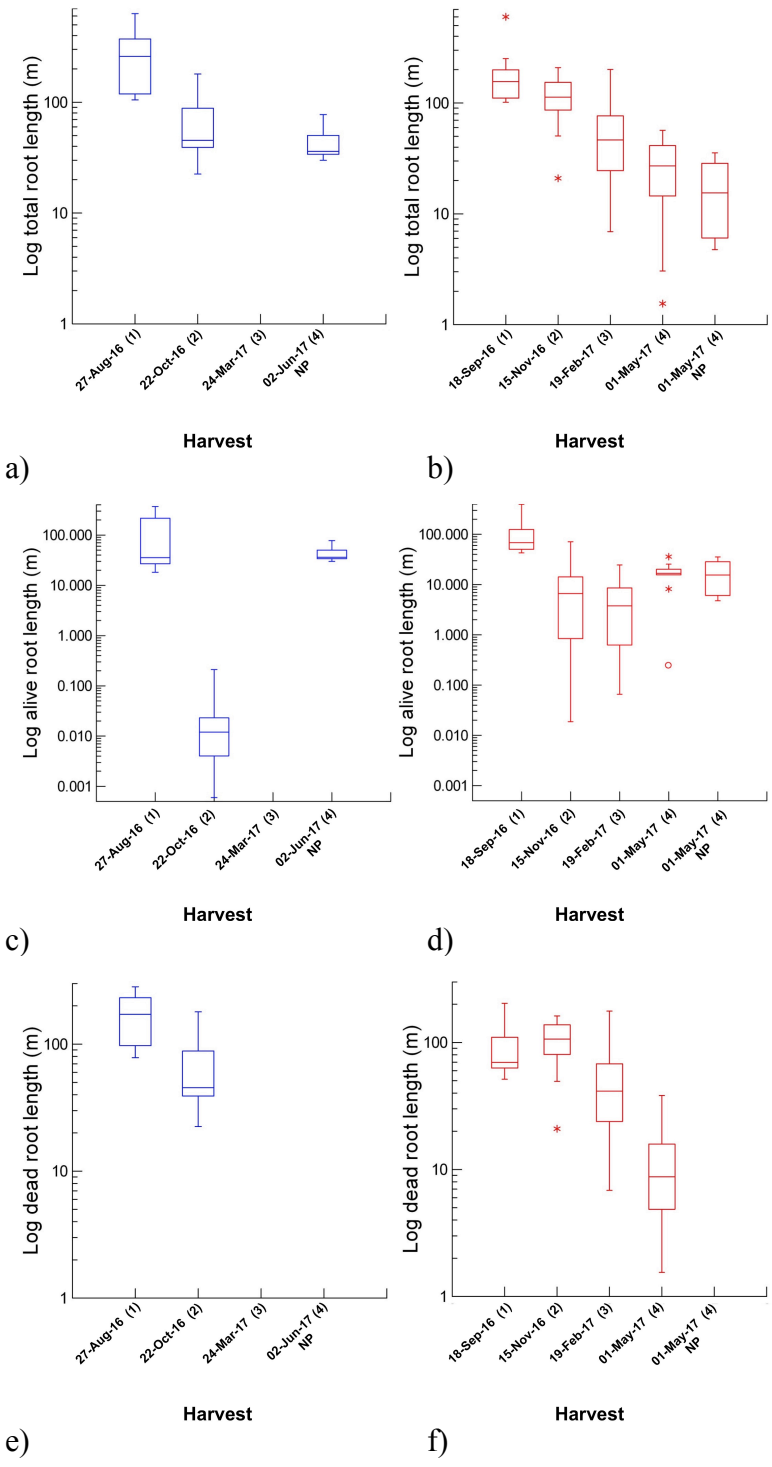

Figure 8. Boxplots for total, living and dead root length of *S. latifolia* in the four harvests in Sudbury, ON (a, c, e) and Gastonia, NC (b, d, f) where NP stands for the new plant material grown in spring of 2017.

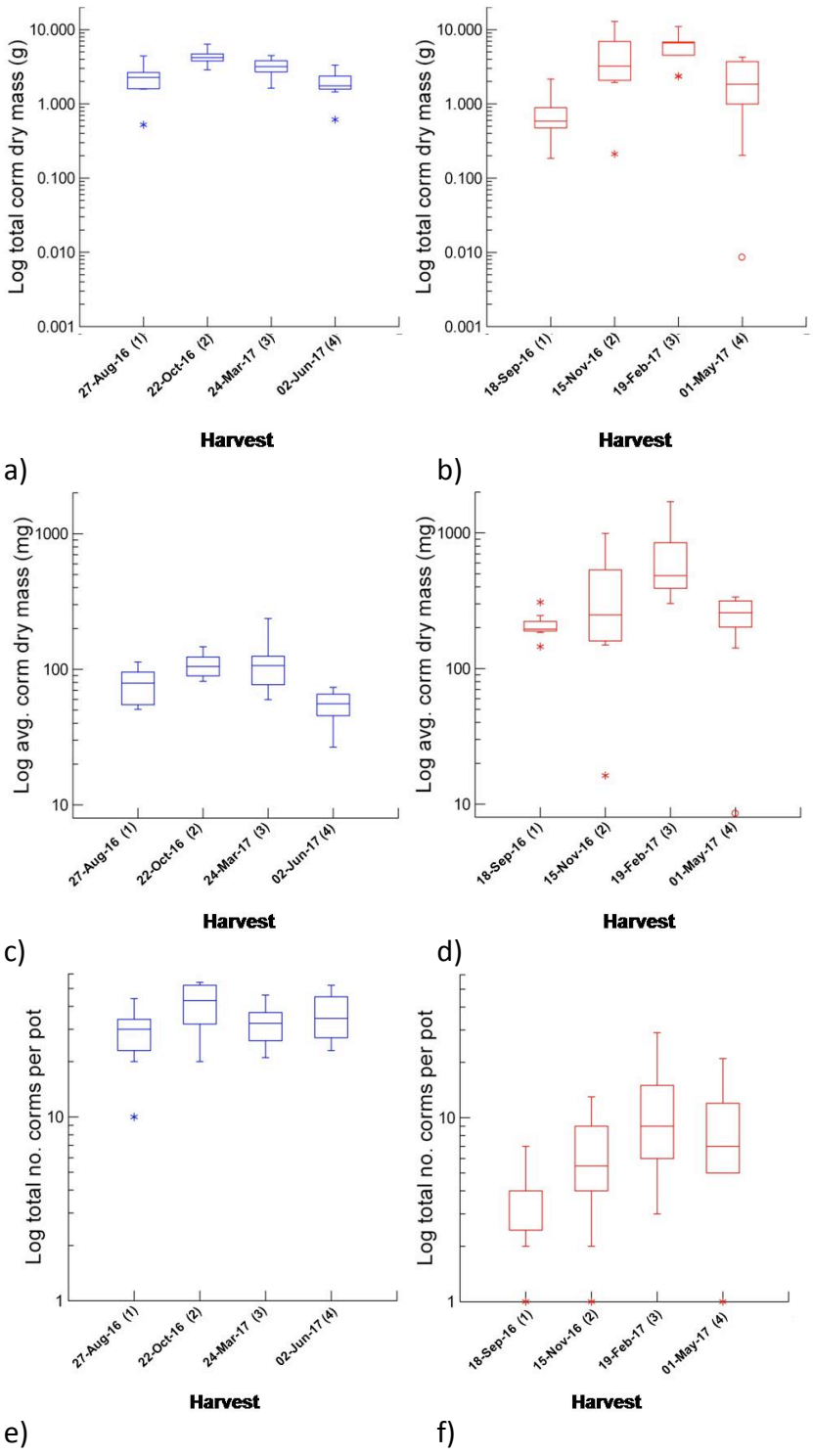

Figure 9. Boxplots for total corm dry mass, average corm dry mass and total number of corms per mesocosm of *S. latifolia* in the four harvests in Sudbury, ON (a, c, e) and Gastonia, NC (b, d, f). All bulbs grew in the growing season of 2016.

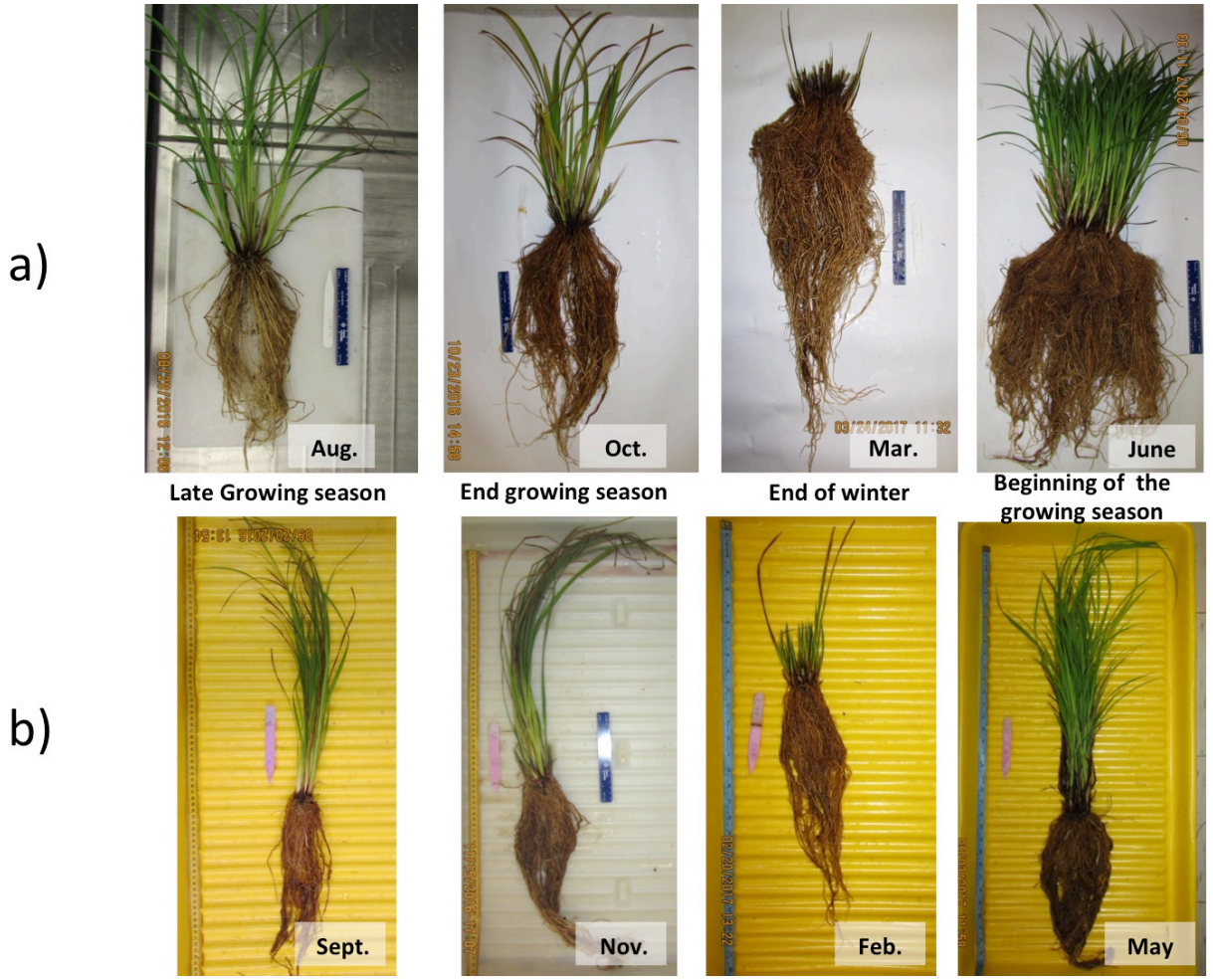

Figure 10. Photos of representative specimens of *C. crinita* at each of the four harvest from late growing season 2017 to early growing season 2017 throughout a full year of plant growth in a) Sudbury, ON and b) Gastonia, NC.

Figure 11. Photos of representative specimens of *S. latifolia* at each of the four harvests from late growing season 2016 to early growing season 2017 throughout a full year of plant growth in a) Sudbury, ON and b) Gastonia, NC.

4 Discussion

The two studied species with contrasting root strategy, overwintering and autumn-senescing roots, previously observed in Northern Ontario (Ryser and Kamminga 2009; Alsahame 2016; SJE Marcotte unpublished data) maintained their respective patterns of biomass turnover also in Gastonia, NC, which has much warmer and much shorter winter. More than 75% of the root system of *C. crinita* remained alive throughout the year while no detectable winter mortality in either location. The roots of *S. latifolia* maintained the autumn-senescing strategy in both locations, however the timing of root senescence differed. Root senescence in Gastonia plants was slower and later in the autumn relative to the fast and complete root senescence in early autumn in Sudbury plants.

4.1 Temperature At Study Sites

Substrate temperatures in Sudbury were slightly above 20°C in July and August, while in Gastonia the substrate temperatures remained at 20°C from May until September and reached values 4-6°C higher. In both locations the length of the actual growing season in 2016 remained within one standard deviation from the long-term averages. The growing season of 2016 in Sudbury was 4 days shorter than the long-term average, whereas in Gastonia it was 8 days longer (N.C. State extension1996; OMAFRA 2016).

During the winter months, Sudbury substrate temperatures remained between 1 and 3°C from December to March, while in Gastonia December and January being the coldest months had temperatures of 7-8°C. Such temperatures could sustain root growth (Alvarez-Uria and Körner 2007). Anderson and McNaughton (1973) reported that along

an elevational gradient cooling soil temperatures down to 3°C had little effect on transpiration and photosynthesis for a variety of herbaceous perennial species, but retarded plant growth. In arctic sedges, it was observed that minimal root growth can take place at temperatures as low as 2°C (Kummerow and Ellis 1984). Aside from the difference between locations, during the coldest months the substrate temperatures in Gastonia were constantly about 1°C higher for *S. latifolia* plants compared to *C. crinita* ones. D. Courchesne, A. Wilson and P. Ryser (unpublished data) reported that in Northern Ontario species with autumn senescing roots were predominantly found in soils with warmer temperatures compared to species with overwintering roots. But besides the difference in substrate temperatures, plant respiration depends on the acclimation and adaptation of the organism to warm or cold environments (Chapin III 1974).

4.2 *Carex Crinita*: Overwintering Roots Strategy

4.2.1 Aboveground traits

C. crinita shoot biomass barely changed in autumn suggesting that the species maximized the utilization of the favourable temperatures during the marginal season, without allocating much biomass above ground aside from that of the winter buds. In both locations the leaves were deciduous and eventually senesced during the winter, only the winter buds remaining alive aboveground. The formation of winter buds in *C. crinita* as we observed is characteristic for many other species of the genus which begin spring growth with shoots formed in autumn or early winter. This enables a rapid growth in early spring since shoots can immediately begin photosynthesizing using the stored nutrients in their rhizomes when it gets warmer (Bernard 1990). Therefore winter bud

formation at the end of the previous growing season and undergoing winter dormancy allows the leaves to maximize the utilization of the productive conditions of the growing season.

4.2.2 Belowground traits

C. crinita, showed more than two-fold increase in the root and rhizome dry masses between harvests 1 and 2 in both study locations at the end of the autumn, and no further growth until spring. This clearly indicates that both roots and rhizome were used in the autumn to store carbon and nutrients for the winter. Since the leafy dry mass showed no change during the time, the increase in belowground dry mass was likely a result of new photosynthesis rather than reallocation of carbohydrates from the shoots to roots and rhizomes. In alpine *Oxytropis sericeae* the peak concentrations of non-structural carbohydrates into perennating organs occurs during the autumn, as a preparation for winter (Wyka 1999). While the stored carbon in *Liquidambar styraciflua* is mostly used to fuel root respiration and only to a lower extent for new growth in spring, which is mainly fuelled by new photosyntates (Lynch *et al.* 2013). The mineral nutrients stored in the roots during late summer and autumn are very well used in spring for new growth, as seen for the arctic sedge *Eriophorum vaginatum* (Chapin III *et al.* 1986) and the perennial weeds *Cichorium intybus* and *Taraxacum officinale* (Cyr *et al*. 1990).

The root survival in both sites remained above 75% at all harvests and corresponds to the overwintering root strategy observed for other *Carex* species (Ryser and Kamminga 2009; Alsahame 2016; Y. Hoogzaad and S.J.E. Marcotte unpublished

data). The roots of Gastonia plants showed almost complete survival throughout the year. Sudbury plants had initially only 75% red-stained roots, but rather than decreasing over the winter, the percentage of stained roots increased over time. In autumn and spring this could be explained by new growth, as observed new growth (albeit not significantly), but it is unlikely during the winter. On the other hand, increment in root survival during the winter could result from decomposition of dead roots, which may have contributed to an increase in this ratio during winter, when the length of dead roots decreased. This would indicate that the observed dead roots in harvest 1 were a result of root mortality in the summer for unknown reasons, and not due to root autumn senescence. Additionally, during the first harvest in Sudbury the vacuum failed partially to exert the usual force to remove the excess air from the root aerenchyma, possibly resulting in a lower root staining. Although in spring in both locations root survival was above 95%, the total and living root lengths remained similar to those measured in the winter. This indicates that the plant remained dormant throughout the winter and resumed growth in early spring as a response to the change in seasonal conditions, as shown by Bernard (1990) and Rohde and Bhalerao (2007).

There was no significant root and rhizome growth in spring in either of the two locations. This suggests that the plants resumed growth first for the shoots since the root system is already established. Also Sloan *et al.* (2016) describes that in arctic sedge communities, root production occurred in early July, about 1 week after the peak of leaf production in late June.

The results obtained in the present study aligned with the predicted high root survival throughout a full year of plant growth. With no sign of increasing root mortality

in early summer, the data indicates that species with overwintering roots have a life span longer than a year (the length of the study) in both locations differing in growing season lengths by over two months.

4.3 *Sagittaria Latifolia:* Autumn-Senescing Roots

4.3.1 Aboveground traits

The aboveground parts of *S. latifolia* senesced and decomposed completely in Sudbury before the winter while in Gastonia this happened during the winter, leaving mostly underground storage units known as corms as the surviving plant parts. In Sudbury the shoots senesced completely in autumn, while in Gastonia at harvest 2 the plants still had about 25% of the shoot biomass alive, senescing gradually. Decreasing latitude is known to delay leaf senescence explaining the later leaf senescence of *S. latifolia* in Gastonia (Kollmann and Bañuelos 2004; Körner 2007; Fracheboud et al. 2009). Similarly, Pilon *et al*. (2002) found for clones of *Potamageton pectinatus* coming from a latitudinal range of 24°N in Egypt to 68°N in Russia, that high latitude genotypes have shorter life cycles than those of lower latitudes. The abnormally high autumn temperatures recorded in September and October with 3-4°C above the long-term averages, may have additionally contributed to the slow leaf senescence in Gastonia. And high temperatures may result in insensitivity to photoperiod length as reported by Heide (2008) for species of the genus *Prunus* in Norway. On the other hand, for some species such as *Persicaria bistorta* senesce occurs after a given period of time, regardless of the length of the growing season (Starr *et al*. 2000).

4.3.2 Belowground traits: roots

S. latifolia roots mostly senesced before the winter in both locations. Alsahame (2016) already reported this pattern for *S. latifolia* in Sudbury. Nevertheless, there was a difference between the locations. For Sudbury plants virtually all roots died by the beginning of the winter before the substrate reached temperatures of 5°C, while at that time for Gastonia plants about 10% of the root length remained alive. This suggests that the species can respond to shorter and less severe winters prolonging its root lifespan. In Sudbury, root senescence was completed before low temperatures would cause tissue damage and plants lose the nutrients. The question remains whether the few surviving roots of *S. latifolia* in Gastonia have any functional significance for the plant in the new growing season. This is at least in theory possible, as in harvests 3 and 4 roots from 2016 in some plants were still connected to the corms and later to the new plants growing in spring 2017 by living stolons. The steles of these roots were still clearly staining red suggesting that they may have had reduced respiration costs, as suggested by Schneider (2017). He found that root cortical senescence in four genotypes of barely, an annual grass, reduced respiration by 87%, possibly elongating root functional life span. *Potamageton pectinatus* and *Sagittaria latifolia* have similar life history, since for both species the plant senesces before the winter except for the propagules from which new plants will emerge the following growing season. However Van Wijk (1988) reported that under mild climatic conditions *P. pectinatus* remains green all year round. The increase in percentage of roots alive in harvest 4 for the cohort of roots of 2016 in spring can be explained by the decomposition and loss of dead roots. Root decomposition in

wetlands is associated with the seasonal variation in temperature (Stein and Hook 2005), and has been found to be high in spring (Wetzel and Howe 1999).

4.3.3 Belowground traits: corms

The corm dry mass increased significantly in autumn indicating storage of carbohydrates for the winter. Gastonia plants had a significantly lower number of corms per plant, but they were much larger compared to Sudbury ones. Verburg and Grava (1998) showed that the trade-off between size and number of propagules in *Circaea luterina,* a woodland pseudo-annual similar to *S. latifolia*, is driven by the environmental conditions to which the plant is subjected. In an environment with milder stress, larger but fewer propagules were found to be more competitive conferring the young plant resources to grow faster (Werner and Platt 1976). As reported by Verburg (1998), the larger hibernacles on *Circaea luteriana* provided more capital with higher initial food reserves resulting in larger plants compared to small hibernacles. Santamaria and Llano-Garcia (2004) reported a positive correlation between increasing latitude (24 °N in Egypt to 68 °N in Russia) and the number of propagules produced in *Potamageton pectinatus*, which increased in number of units but with smaller mass as latitude increased. Such a latitudinal variation was also observed in *S. latifolia* in the present study. However, besides the ecological reasons, the variation in average corm size is also influenced by the sexual system, monoecious populations of *S. latifolia* have more but smaller corms compared to diecious populations (Dorken and Barrett 2003). As the sexual systems of the populations of *S. latifolia* in the present study were unknown, the difference in corm size could have been attributed to a difference in the sexual system.

4.4 Ecological Significance of Two Strategies

Then what are the adaptive advantages of these two root turnover strategies? In Northern Ontario, perennial wetland monocot species with overwintering roots are characterized by having a higher leaf and root dry matter content than species with autumn-senescing roots (Gagnon 2014b). High root dry matter content is known to be associated with robust tissue characterized by small and thick cell walls and high proportion of sclerenchymatic tissue (Wahl and Ryser 2000). Wright *et al*. (2004) described that longer leaf lifespan is associated with a more robust construction in the form of a higher leaf dry mass per leaf area. Furthermore, leaf lifespan was related to leaf economics by the duration over which photosynthetic revenue is returned over the cost of dry mass invested to build new leaf area. The two root turnover strategies seem to fit into the described economics gradient. However the dichotomous nature of their strategies present additional information to consider understanding the adaptive value of root lifespan along environmental gradients. Nevertheless, the advantages of annually roots are still inconclusive, further studies are required to expand the knowledge on this strategy.

Variation in biomass turnover patterns along the leaf economic spectrum and the dichotomous distinction of the strategies of deciduous and evergreen leaves affect greatly the global carbon cycle (Keeling *et al.* 1996). Similarly, it is important to understand the root economic spectrum associated with root phenology and overwintering strategies of perennial wetland vegetation. Studying the response of species with contrasting root turnover strategies to changes in the growing season length will help us to understand how the shifts in community composition will affect ecosystem processes such as carbon

and nutrient cycles (Wookey *et al* 2009). The two species, *C. crinita* and *S. latifolia,* retained their respective root turnover strategies, overwintering roots and autumn-senescing roots, in both locations in spite of the difference in growing season length and in harshness of the winter. This indicates that the root strategies are species specific, maintained to a similar manner to that observed in deciduous and evergreen leaves throughout the species' range of distribution.

For both species the aboveground biomass is deciduous, and the difference in root turnover patterns further emphasizes that belowground phenology does not necessarily match the aboveground phenology. The slow root mortality of *S. latifolia* in Gastonia, but close-to-complete mortality until the new growing season, indicates that although the species can adapt to a less severe winter, its strategy still remains as a fast-growing species with annually renewed roots. The surviving roots in the Gastonia genotype, although only a small percentage, indicated that decreasing day length might not be the main trigger of root senescence. In contrast, the Sudbury genotype senesced completely before the frosts, most likely triggered by shorter days as an adaptation to minimize the likelihood of resource loss due to frost damage (Zepeda-Gómez and Lot 2005).

5 Conclusion

In conclusion, the data indicates that the two root strategies, overwintering and autumn-senescing roots, are species specific and are maintained across a varying growing season length. *C. crinita* with overwintering roots showed overall high root survival in both locations, however data did not provide conclusive evidence of higher winter root mortality in the southern location as hypothesized. The species with autumn-senescing roots, *S. latifolia*, had a slower root turnover in the southern location. This was partially a result of the difference in growing season length resulting in a longer root lifespan for the southern genotype compared to the northern one. Additionally, some roots did not senesce at all surviving the winter, indicating that the seasonal nature of the leaf lifespan in the northern location is slightly modified when the winter is shorter and warmer. Nevertheless, the species still retained its strategy of renewing its roots annually, in contrast to the species with overwintering roots.

Future questions of interest along the same line of research would address: (1) How long do roots of *C. crinita* survive beyond the first winter and to what extent is root mortality of the two genotypes depending on winter temperatures?, (2) What is the functional significance of the few roots surviving the winter in the southern location for *S. latifolia*?, and (3) What happens with the root life span of species with autumn-senescing roots if the winter is shorter and warmer? Since root biomass can account for more than half of the biomass production in northern wetlands, expanding the knowledge on the patterns and strategies of root phenology will provide a better understanding of how the changing climate can influence species distribution, abundance and the effects of these on the carbon cycle.

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Appendices

Appendix 1. Summary results for the soil analysis results of Total Kjeldahl nitrogen and BCSALM extraction on the playsand and topsoil (Timberland) used in Gastonia and the topsoil (Bainbridge) used in Sudbury (Testmark Laboratories, Sudbury, ON).

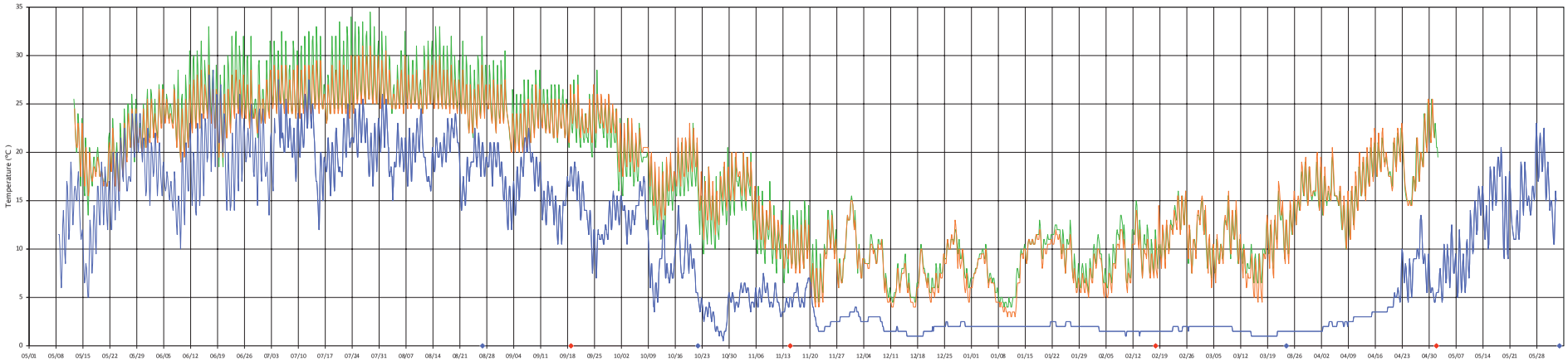
Soil Type	mg/kg	μg/g			
	TKN	Ca	Fe	Mg	P
Playsand	-	59	5040	29.4	4.9
Topsoil (Timberland)	678	4220	7790	1190	430
Topsoil (Bainbridge)	2020	2730	6740	1315	525

Appendix 2. Summary results for the soil analysis by Mehlich-3 Extraction (NCDA & CS) on the natural wetland soil in Gastonia, NC for the *C. crinita* and *S. latifolia* trench at each harvest (H1-H4).

C. crinita	pH	HM	mg/L								
		%	P^{-3}	K^{+}	Ca^{+2}	Mg^{+2}	S^{-2}	Na^{+}	Mn^{+2}	Cu^{+}	Zn^{+2}
H1	4.7	0.76	9	44	541	56	61	0.2	57.8	1.6	11.6
H2	4.8	0.51	5	35	563	58	48	0.2	44.4	2.6	13.1
H3	4.9	0.51	8	35	607	55	38	0.1	61.6	1.3	11.8
H4	4.8	0.36	13	33	569	55	46	0.2	66.4	1.5	11.3
Average	4.8	0.54	9	37	570	56	48	0.2	57.6	1.8	11.9
S. latifolia											
H1	4.9	0.71	7	51	687	74	45	0.2	44.9	1.4	11.5
H2	4.8	0.46	6	36	578	63	30	0.2	30.8	1.4	11.6
H3	5	0.41	9	46	742	80	34	0.1	54.1	0.9	11.5
H4	4.9	0.36	13	47	679	71	38	0.2	51.6	1.4	12.4
Average	4.9	0.49	9	45	672	72	37	0.2	45.4	1.3	11.8

Appendix 3. Summary results for the water analysis of the creek water adjacent to the trenches in Gastonia, NC with pH, cations, total organic carbon (NPOC) and total nitrogen.

Units	Na^{+}	NH_4^{+}	K^{+}	Mg^{+2}	Ca^{+2}	NPOC	TN	pH
µg/g	7.822	3.1816	3.2197	4.7961	17.0563	-	-	4.9
mg/L	-	-	-	-	-	33.23	4.16	



Appendix 4. Substrate temperatures recorded inside the mesocosms in Sudbury, ON (blue line) and Gastonia, NC (yellow line – *C. crinita* trench and orange line – *S. latifolia* trench) during the time of the experiment from May 2016 to June 2017. The marked dates along the X-axis refer to the harvests, blue for Sudbury, ON and red for Gastonia, NC.

Appendix 5. Average monthly soil temperature and monthly cumulative growing degree-days (GDD) for *C. crinita* and *S. latifolia* from September 2016 until August 2017 along the northern and southern shores of Vermilion River in Sudbury, ON. The values are the averages of ten data loggers, two per plant species in five sites for except for *S. latfolia* with nine data loggers.

Shore	*Carex crinita* South		North	
Month	Temperature (°C)	GDD	Temperature (°C)	GDD
September	13.8	277	16.4	925
October	10.3	340	12.1	1107
November	5.4	173	6.5	576
December	2.8	93	2.3	201
January	2.0	66	1.3	116
February	1.4	41	0.9	79
March	1.2	40	1.0	82
April	3.4	109	3.7	319
May	9.0	295	10.1	911
June	13.5	440	16.4	1440
July	15.3	504	18.2	1649
August	15.3	289	17.9	940
	Sagittaria latifolia			
September	15.3	308	17.6	356
October	12.4	409	13.7	450
November	6.4	205	6.9	221
December	2.9	97	2.8	92
January	1.5	49	1.4	45
February	1.2	36	1.1	32
March	1.3	42	1.1	37
April	3.6	115	5.3	371
May	10.0	330	11.7	476
June	16.6	529	17.0	543
July	19.1	629	19.7	650
August	19.3	362	20.4	383

Appendix 6. Average monthly soil temperature and monthly cumulative growing degree-days (GDD) for *C.crinita* and *S. latifolia* from May 2017 to January 2018 in Gastonia, NC. Temperatures were recorded inside the mesocosms substrate and 10 cm depth in the natural trench substrate where the mesocosms with plants were placed. The values are the averages of two dataloggers in a mesocosms and two in the natural trench substrate.

Position	*Carex crinita* Mesocosms		Trench substrate	
Month	Temperature (°C)	GDD	Temperature (°C)	GDD
May	21.3	739	21.3	713
June	24.2	725	23.4	701
July	24.2	751	23.6	731
August	22.3	696	22.4	699
September	18.9	566	19.5	585
October	15.8	491	17.0	528
November	10.6	320	12.0	363
December	7.1	221	8.4	260
January	2.4	67	4.1	114
	Sagittaria latifolia			
May	21.6	752	20.9	706
June	25.7	769	23.2	694
July	25.8	801	24.4	757
August	22.8	711	22.6	703
September	19.2	575	19.4	580
October	16.3	505	17.1	531
November	10.9	330	12.2	367
December	7.7	238	8.7	269
January	3.4	95	4.8	131

Appendix 7. Summary results the general linear model on the effect of flowering on various traits in *C. crinita* in Sudbury. Independent variables are plant type (plants with vegetative shoots only and plants with both vegetative and flowering shoots) and harvest.

Dependent factor	Independent factors	*n*	R^2	F-ratio	*P*-value
Shoot dry mass	Harvest	38	0.657	13.2	<0.001***
	Plant type			3.8	0.061
	Harvest × PT			0.1	0.955
Rhizome dry mass	Harvest	38	0.649	17.3	<0.001***
	Plant type			1.7	0.203
	Harvest × PT			0.3	0.802
Root dry mass	Harvest	38	0.440	6.8	0.001**
	Plant type			2.4	0.135
	Harvest × PT			0.1	0.954
Total root length	Harvest	38	0.314	2.9	0.048*
	Plant type			1.9	0.183
	Harvest × PT			0.4	0.789
% Roots alive	Harvest	38	0.840	50.9	<0.001***
	Plant type			1.6	0.221
	Harvest × PT			0.7	0.535
Alive root length	Harvest	38	0.340	3.7	0.022*
	Plant type			1.6	0.215
	Harvest × PT			0.3	0.820
Dead root length	Harvest	38	0.558	10.4	<0.001***
	Plant type			3.3	0.079
	Harvest × PT			0.5	0.677
Specific root length	Harvest	38	0.675	16.5	<0.001***
	Plant type			0.2	0.676
	Harvest × PT			0.5	0.661

Appendix 8. Summary of means (±1 standard error) based on plant type for each plant trait measured in *Carex crinita* in Sudbury, where GS means growing season.

Trait	Shoot dry mass (g)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	5.635	6.774	1.761	8.214	3.742	5.753	0.903	6.550
S. E.	0.808	1.345	1.103	1.295	0.904	1.016	0.186	1.201
Trait	Rhizome dry mass (g)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	0.574	3.412	3.409	4.752	0.464	2.207	2.953	3.398
S. E.	0.045	1.214	2.592	0.553	0.031	0.435	0.583	0.505
Trait	Root dry mass (g)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	6.831	16.18	14.18	17.09	3.808	12.27	8.997	15.80
S. E.	1.414	2.728	11.59	2.157	1.147	2.957	1.860	3.866
Trait	Total root length (m)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	466.9	734.6	634.5	618.3	259.0	557.3	255.5	680.0
S. E.	97.52	133.9	577.2	70.23	72.54	143.1	54.45	211.4

To be continued

Appendix 8. Continuation

Trait	%Root alive							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	75.02	78.61	86.60	96.27	77.22	82.83	86.02	96.58
S. E.	2.096	1.756	1.457	0.494	2.393	1.819	1.121	0.422
Trait	Alive root length (m)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	343.1	585.6	557.9	596.3	206.8	462.1	217.9	657.9
S. E.	64.41	118.2	509.1	69.83	60.61	117.4	45.03	204.3
Trait	Dead root length (m)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	123.8	149.0	76.58	22.01	52.28	95.22	37.56	22.03
S. E.	33.80	18.58	68.07	1.965	12.09	28.83	9.899	7.271
Trait	Specific root length (m/g)							
Plant type	Vegetative shoots				veg. shoots + flower shoots			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	5	5	2	5	5	5	6	5
Mean	58.14	45.32	34.59	36.48	55.04	44.65	29.37	40.63
S. E.	1.836	2.094	12.42	1.472	2.611	5.212	2.244	3.234

Appendix 9. Summary of means (±1 standard error) and Tukey's difference test following the GLMs of table 8 for the various plant traits measured in *Carex crinita* in Sudbury, ON and Gastonia, NC where GS means growing season. Tukey's difference tests were conducted for harvest (H), location (L) and interaction (I) factors according to the significance obtained in the GLM. Relevant comparisons for the interaction effect were considered between harvests of the same locations and the matching harvest in the two locations.

Trait	Shoot dry mass (g)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	4.689	6.263	1.118	7.382	3.275	4.357	1.616	9.606
S. E.	0.653	0.813	0.286	0.878	0.329	0.525	0.170	0.629
Tukey's test – I	a	a	b	ac	ab	a	b	c
Trait	Rhizome dry mass (g)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	0.519	2.809	3.067	4.075	1.175	2.426	2.685	2.936
S. E.	0.032	0.640	0.654	0.419	0.101	0.325	0.366	0.512
Tukey's test – I	a	b	b	b	a	ab	b	b
Trait	Root dry mass (g)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	5.320	14.23	10.29	16.44	3.520	7.811	7.007	10.02
S. E.	0.995	2.005	2.715	2.098	0.363	0.750	0.966	0.735
Tukey's test - H	a	b	bc	bd	§	§	§	§
Tukey's test – L	a			>			b	

To be continued

Appendix 9. Continuation

Trait	Total root length (m)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	362.9	645.9	350.2	649.1	333.2	432.2	309.8	313.3
S. E.	66.96	96.98	131.7	105.5	64.52	47.65	40.47	14.89
Tukey's test - H	a	ab	ac	ab	§	§	§	§
Tukey's test – L			a				a	
Trait	% Roots alive							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	76.12	80.72	86.17	96.43	94.44	94.78	91.84	97.48
S. E.	1.544	1.384	0.871	0.311	0.513	0.349	0.656	0.164
Tukey's test - I	a	b	c	d	e	e	ef	deg
Trait	Alive root length (m)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	274.9	523.9	302.9	627.1	315.6	410.3	284.1	305.4
S. E.	47.49	81.19	115.9	102.3	61.98	45.41	37.06	14.39
Tukey's test -H	a	b	a	b	§	§	§	§
Tukey's test – L			a				a	
Trait	Dead root length (m)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	88.05	122.1	47.32	22.02	17.62	21.95	25.75	7.944
S. E.	20.70	18.49	16.09	3.551	2.976	2.580	4.242	0.701
Tukey's test – I	a	ab	ac	cd	e	ef	acef	eg

To be continued

Appendix 9. Continuation

Trait	Specific root length (m/g)							
Site	Sudbury, ON				Gastonia, NC			
Harvest	1	2	3	4	1	2	3	4
Season	Late GS	End GS	End winter	Beginning GS	Late GS	End GS	End winter	Beginning GS
N	10	10	8	10	10	10	10	10
Mean	56.59	44.98	30.67	38.56	92.95	56.59	48.32	32.16
S. E.	1.591	2.650	2.990	1.813	11.96	4.850	7.933	1.822
Tukey's test – I	a	ac	b	bc	d	ace	ef	bcf

§ Tukey's difference test was only conducted for harvest to indicating the difference between harvests 1 to 4 in each location. To avoid confusion and repetition the result was placed under Sudbury but the differences applies for both locations.

Appendix 10. Summary of means (±1 standard error) and Tukey's difference test by locations and harvest for the traits measured in *Sagittaria latifolia* in Sudbury, ON and Gastonia, NC where GS means growing season. Tukey's test of difference was conducted for harvest (H), location (L) and interaction (I) factors according to the significance obtained in the GLM. Relevant comparisons for the interaction effect were considered between sites for matching harvests and between harvests within each site. Comparisons for all plant traits except for % Roots alive and bulb measurements are as follow: comparison between harvests 1-2 in both locations, harvests 1-4 in Gastonia for plant material belonging to the growing season of 2016 and harvests 4 between locations. The bulb measurements were compared between harvests of the same locations and the matching harvest in the two locations. The % Roots alive was tested with a non-parametric Kruskal-Wallis and differences are based on the rank sums obtained.

Trait	Shoot dry mass (g)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	.	10
Mean	3.887	0.001	0	0.619	3.773	0.893	0	.	0.507
S. E.	0.362	0.000	0	0.076	0.996	0.197	0	.	0.101
Tukey's test – I	a	b	.	.	a	c	.	.	.
Tukey's test – L	a<		.	c	b		.	.	c
Trait	Stolon dry mass (g)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	10
Mean	0.966	0.228	0	0.123	0.560	0.249	0.093	0	0.197
S. E.	0.115	0.023	0	0.016	0.066	0.035	0.020	0	0.053
Tukey's test – I	a	b	.	.	c	b	.	.	.
Tukey's test – H	.	.	.	.	c	b	d	.	.
Tukey's test – L	a		.	b	a		.	.	b

To be continued

Appendix 10. Continuation

Trait	Root dry mass (g)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	9	10	10
Mean	1.228	0.319	0	0.241	1.107	0.980	0.606	0.306	0.142
S. E.	0.208	0.076	0	0.035	0.187	0.129	0.184	0.063	0.033
Tukey's test – I	a	b	.	.	a	a	.	.	.
Tukey's test – H	.	.	.	.	a	a	ab	b	.
Tukey's test – L	a<		.	c>	b		.	.	d

Trait	Total root length (m)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	10
Mean	272.5	68.34	0	44.98	200.4	115.7	62.25	27.84	18.24
S. E.	53.06	15.12	0	5.370	47.29	18.37	17.60	5.999	3.730
Tukey's test – I	a	b	.	.	a	ab	.	.	.
Tukey's test – H	.	.	.	.	a	ab	bc	c	.
Tukey's test – L	a		.	b >	a		.	.	c

Trait	% Roots alive								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	10
Mean	31.34	0.026	0	100	50.79	8.194	8.771	48.47	100
S. E.	5.922	0.011	0	0	3.452	3.299	2.665	8.488	0
Kruskal-Wallis H	.	.	.	.	a	b	b	a	.
Kruskal-Wallis – L	a <	.	.	c	b	.	.	.	c

To be continued

Appendix 10. Continuation

Trait	Alive root length (m)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	10
Mean	104.9	0.030	0	44.98	111.4	13.62	6.130	15.65	18.24
S. E.	38.14	0.020	0	5.37	34.13	6.876	2.388	3.454	3.730
Tukey's test – I	a	b	.	.	a	c	.	.	.
Tukey's test – H	.	.	.	.	a	c	c	ac	.
Tukey's test – L	a<		.	c >	b		.	.	d

Trait	Dead root length (m)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	10
Mean	167.6	68.31	0	0	89.03	102.1	56.12	12.19	0
S. E.	23.87	15.11	0	0	14.32	14.05	15.66	3.472	0
Tukey's test – I	a	b	.	.	a	a	.	.	.
Tukey's test – H	.	.	.	.	a	a	a	c	.
Tukey's test – L	a		.	b	a		.	.	b

Trait	Specific root length (m/g)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	10
Mean	214.6	228.4	0	192.9	175.2	116.3	126.5	90.68	138.4
S. E.	13.76	18.82	0	7.440	15.69	7.358	15.08	4.173	13.76
Tukey's test – I	a	a	.	.	ab	c	.	.	.
Tukey's test – H	.	.	.	.	ab	c	ac	c	.
Tukey's test – L	a>		.	d	b		.	.	d

To be continued

Appendix 10. Continuation

Trait	Total corm dry mass (g)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	.
Mean	2.296	4.386	3.163	1.97	0.569	4.916	5.424	2.146	.
S. E.	0.346	0.333	0.293	0.252	0.209	1.255	1.044	0.496	.
Tukey's test – I	a	a	a	a	ab	ac	acd	abc	.

Trait	Average corm dry mass per plant (mg)								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	7	10	9	10	.
Mean	78.48	109.4	112.7	54.14	210.7	378.2	703.3	232.8	.
S. E.	6.967	7.465	15.75	4.60	19.79	96.50	148.7	31.49	.
Tukey's test – I	a	a	a	a	ab	ab	c	ab	.
Tukey's test – L	a			<			b		.

Trait	Total number of corms per plant								
Site	Sudbury, ON				Gastonia, NC				
Harvest	1	2	3	4	1	2	3	4	4
Season	Late GS	End GS	End winter	Beginning GS-2017	Late GS	End GS	End winter	Beginning GS-2016	Beginning GS-2017
N	10	10	10	10	10	10	10	10	.
Mean	28.60	41.50	32.90	35.80	2.500	6.200	9.900	8.300	.
S. E.	3.074	3.550	2.567	3.083	0.734	1.191	2.620	1.892	.
Tukey's test – H	a	b	b	ab	§	§	§	§	.
Tukey'ss test – L	a			>			b		

§ Tukey's difference test was only conducted for harvest to indicating the difference between harvests 1 to 4 in each location. To avoid confusion and repetition the result was placed under Sudbury but the differences applies for both locations.